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Unusual pathways and enzymes of central carbohydrate metabolism in Archaea

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Sugar-utilizing hyperthermophilic and halophilic Archaea degrade glucose and glucose polymers to acetate or to CO_2 using O_2, nitrate, sulfur or sulfate as electron acceptors. Comparative analyses of glycolytic pathways in these organisms indicate a variety of differences from the classical Emden–Meyerhof and Entner–Doudoroff pathways that are operative in Bacteria and Eukarya, respectively. The archaeal pathways are characterized by the presence of numerous novel enzymes and enzyme families that catalyze, for example, the phosphorylation of glucose and of fructose 6-phosphate, the isomerization of glucose 6-phosphate, the cleavage of fructose 1,6-bisphosphate, the oxidation of glyceraldehyde 3-phosphate and the conversion of acetyl-CoA to acetate. Recent major advances in deciphering the complexity of archaeal central carbohydrate metabolism were gained by combination of classical biochemical and genomic-based approaches.

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Introduction

Comparative biochemical studies on central carbohydrate metabolism revealed that Archaea utilize modifications of the classical Embden–Meyerhof (EM) and Entner–Doudoroff (ED) pathways for glycolysis (Figures 1–3).

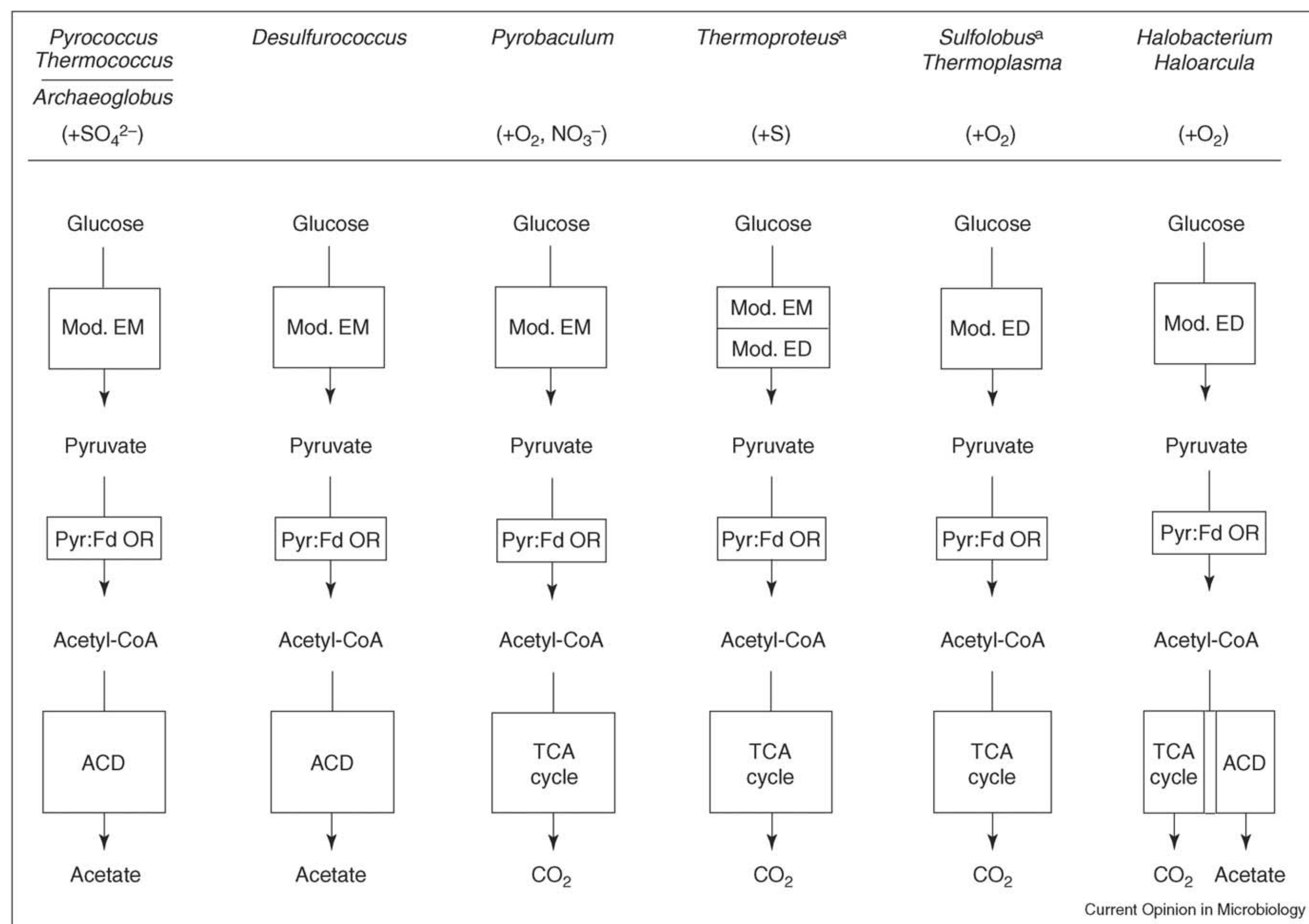
In the classical EM pathway, glucose is converted to fructose-1,6-bisphosphate (FBP), the central intermediate, by way of ATP-dependent hexokinase/glucokinase, phosphoglucose isomerase and ATP-dependent allosteric phosphofructokinases. Cleavage of FBP to dihydroxyacetone phosphate and glyceraldehyde-3-phosphate (GAP) by FBP aldolase and the subsequent isomerization by triosephosphate isomerase yields two mol GAP, which are oxidized to 3-phosphoglycerate by way of phosphorylative glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate kinase; in the latter reaction, ATP is formed by substrate level phosphorylation. 3-Phosphoglycerate is converted to phosphoenolpyruvate (PEP) by way of phosphoglycerate mutase and enolase. The conversion of PEP to pyruvate is catalyzed by allosteric regulated pyruvate kinase, and ATP is formed by substrate level phosphorylation. The net ATP yield of the EM pathway is 2 mol ATP/mol glucose.

In the classical phosphorylative ED pathway, glucose-6-phosphate, which is formed by ATP-dependent glucokinase, is oxidized to 6-phosphogluconate by glucose-6-phosphate dehydrogenase. Subsequent dehydration by 6-phosphogluconate dehydratase yields 2-keto-3-deoxy-(6-phospho)-gluconate (KDPG), the characteristic intermediate of the pathway. KDPG cleavage by KDPG aldolase forms pyruvate and GAP, which is converted to pyruvate by the enzymes also used in the EM pathway. The net ATP yield of ED pathway is 1 mol ATP/mol gucose.

In the hyperthermophilic and the thermophilic aerobic Archaea (*Thermoplasma acidophilum* [1] and *Sulfolobus solfataricus* [2,3,4•], respectively) glucose is metabolized by way of modifications of the ED pathway, whereas the hyperthermophilic fermentative anaerobes *Pyrococcus furiosus*, *Thermococcus* species, *Desulfurococcus amylolyticus*, the sulfate reducer *Archaeoglobus fulgidus* strain 7324 and the microaerophilic *Pyrobaculum aerophilum* use different modifications of the EM pathway ([5–8]; Reher *et al.* personal communication). To date, the only Archaeon known to use modifications of both the EM and ED pathways in parallel for glucose degradation is the hyperthermophilic sulfur-dependent anaerobe *Thermoproteus tenax* [4•,6,9–11,12•]. In aerobic halophilic Archaea (e.g. *Haloarcula marismortui* and *Halobacterium saccharovorum*), ^{13}C-nuclear magnetic resonance (^{13}C-NMR) and enzymatic studies as well as DNA microarray analyses revealed that glucose is degraded only by way of a modified 'semi-phosphorylative' ED pathway [13–15], whereas fructose is almost completely metabolized by way of a modified EM pathway (in *Haloarcula marismortui* [13] and *Haloarcula vallismortis* [16]).

Degradation of pyuvate formed by the various glycolytic pathways involves oxidation to acetyl-CoA, which is catalyzed in all Archaea by pyruvate-ferredoxin oxidoreductase. In anaerobic fermentative Archaea, acetyl-CoA is further converted to acetate by an unusual prokaryotic

Figure 1



Pathways and enzymes of glucose degradation to acetate or to CO_2 in Archaea. *Pyrococcus furiosus*, *Thermococcus celer*, *Desulfurococcus amylolyticus* and *Archaeoglobus fulgidus* strain 7324 (+ SO_4^{2-}) convert glucose to generate acetate as a main product. However, *Pyrobaculum aerophilum* (+ O_2 or NO_3^-), *Thermoproteus tenax* (+ S), *Sulfolobus solfataricus* (+ O_2) and *Thermoplasma acidophilum* (+ O_2) completely oxidize glucose to CO_2 using the external electron acceptors indicated. The aerobic *Halobacterium saccharovorum* and *Haloarcula marismortui* form significant amounts of acetate in addition to CO_2. Glucose degradation to pyruvate proceeds either by modified Embden–Meyerhof pathways (Mod. EM) or by modified Entner–Doudoroff pathways (Mod. ED). The modifications of the EM and ED pathways are specified in Figure 2 and Figure 3, respectively. Pyruvate is converted to acetyl-CoA by pyruvate:ferredoxin oxidoreductase (Pyr:Fd OR). The conversion of acetyl-CoA to acetate is catalyzed by ADP-forming acetyl-CoA synthetase (ADP-forming) (ACD) (acetyl-CoA + ADP + P_i = acetate + ATP + CoA). The oxidation of acetyl-CoA to $2CO_2$ proceeds via the tricarboxylic acid cycle (TCA cycle). [1]*T. tenax* and *S. solfataricus* use a 'branched' (non- and semi-phosphorylative) ED modification (see text).

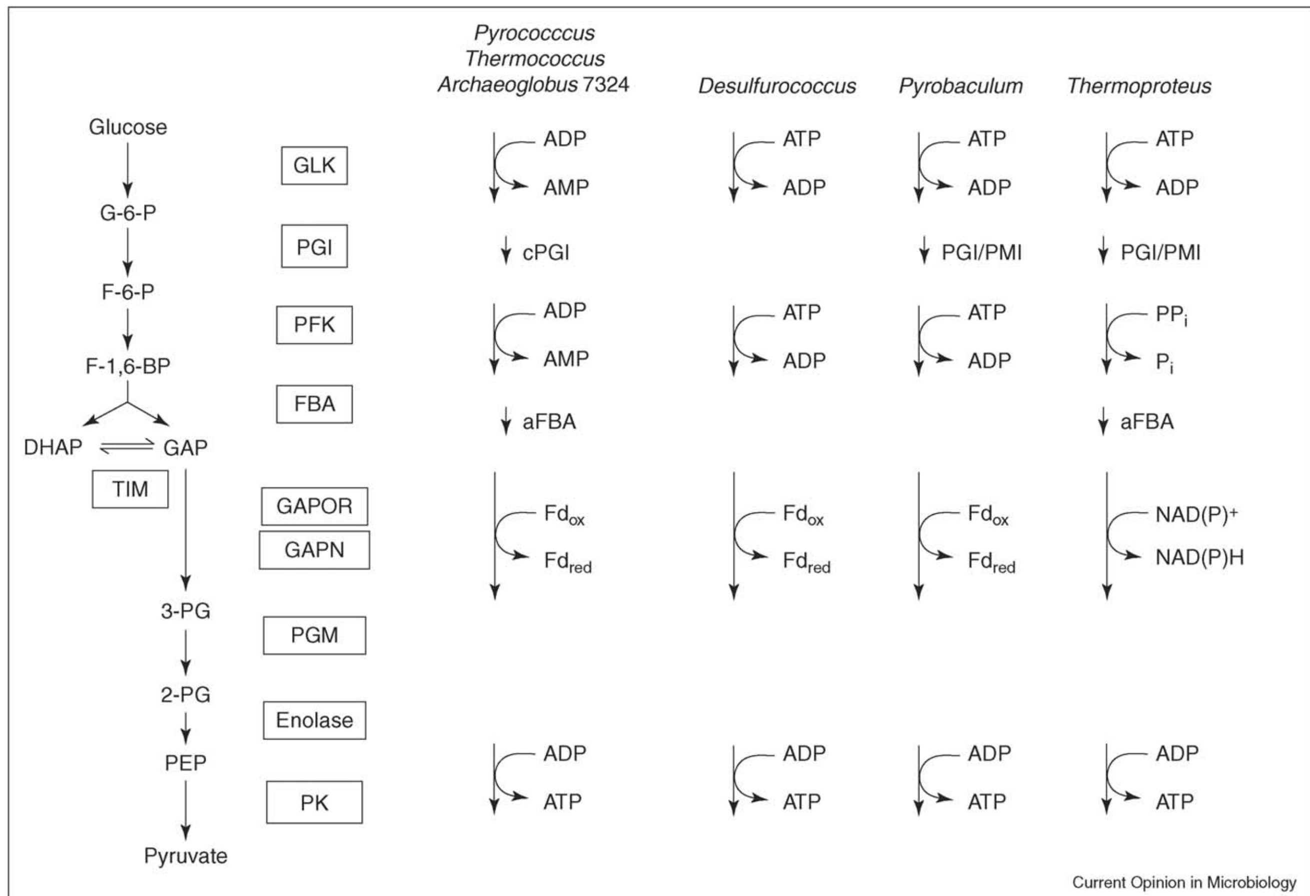
enzyme — ADP-forming acetyl-CoA synthetase (ACD) — whereas in O_2-, nitrate- and sulfur-reducing Archaea, acetyl-CoA is oxidized to $2CO_2$ through the tricarboxylic acid cycle (Figure 1).

Analysis of sugar metabolism in methanogenic Archaea, which are mostly lithoautotrophic H_2/CO_2- or acetate-utilizing organisms, mainly concerns gluconeogenesis. In some *Methanococcus* species, the degradation of intracellular glycogen seems to be performed by glycolysis that uses enzymes of a modified EM pathway.

Although most of the catalyzed reactions, and thus the intermediates of the modified archaeal EM and ED pathways, correspond to the classical glycolytic pathways, most of the respective archaeal enzymes show no similarity to their 'classical' bacterial and eukaryal counterparts and therefore represent examples of non-homologous gene displacement. Furthermore, the great variety of alternative enzymes, often from different enzyme families, identified in different Archaea (e.g. sugar kinases, phosphoglucose isomerases and enzymes of glyceraldehyde-3-phosphate oxidation) reflects a great metabolic diversity in this third domain of life, which exceeds that of Bacteria and Eukarya.

Recently, great advances were obtained in elucidation of archaeal sugar metabolism for enzymes from (hyper)ther-

Figure 2



Modifications of the Embden–Meyerhof (EM) pathway in Archaea. The modified archaeal EM pathways differ from the classical EM pathway as it has unusual enzymes for glucose phosphorylation, phosphoglucose isomerization, fructose 6-phosphate phosphorylation, fructose-1,6-bisphosphate cleavage, glyceraldehyde-3-phosphate oxidation and PEP conversion to pyruvate. For characterized archaeal enzymes, comparison to 'classical' EM enzymes and energy yield, see main body of text. Abbreviations: aFBA, archaeal class I FBA; cPGI, cupin PGI; DHAP, dihydroxyacetone phosphate; FBA, fructose 1,6-bisphosphatae aldolase; F-1,6-BP, fructose 1,6-bisphosphate; Fd_{ox} and Fd_{red}, oxidized and reduced ferredoxin; F-6-P, fructose-6-phosphate; GAP, glyceraldehyde-3-phosphate; GAPN, non-phosphorylative glyceraldehyde 3-phosphate dehydrogenase; GAPOR, glyceraldehyde-3 phosphate-ferredoxin oxidoreductase; GLK, glucokinase (ADP- or ATP-dependent); G-6-P, glucose-6-phosphate; PEP, phosphoenolpyruvate; PFK, 6-phosphofructokinase; 2-PG, 2-phosphoglycerate; 3-PG, 3-phosphoglycerate; PGI/PMI, bifunctional phosphoglucose/phosphomannose isomerase); PGI, phosphoglucose isomerase; PGM, phosphoglycerate mutase; PK, pyruvate kinase; TIM, triosephosphate isomerase.

mophilic Archaea, because most can be expressed in the mesophilic host *Escherichia coli* and can subsequently be characterized as recombinant enzymes. Furthermore, the increased intrinsic rigidity of hyperthermophilic proteins obviously favours crystallization and thus structural analysis. However, in halophiles, the adaptation of proteins to high salt concentrations hampers respective developments and therefore less information is available about purified and crystallized enzymes.

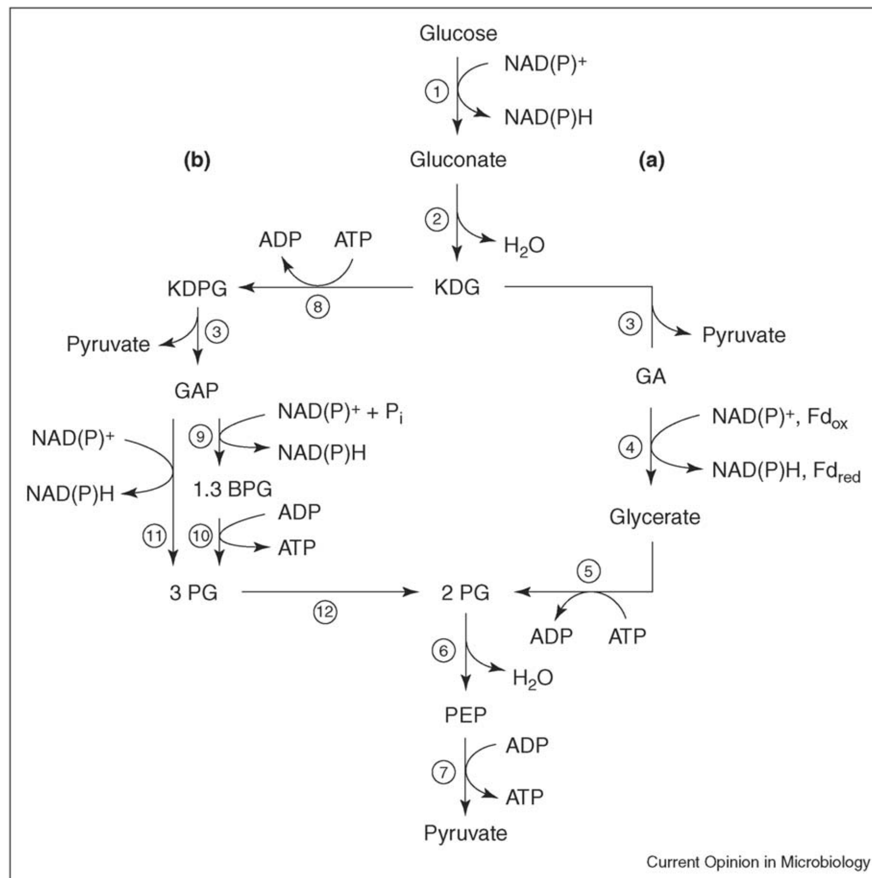
In this review, we will present a survey of the archaeal modifications of EM and ED pathways and of the biochemistry and regulation of the unusual enzymes involved. For previous reviews and review-like articles on this topic, see [4•,6,12•,17,18•,19,20–22].

Unusual enzymes of modfied EM pathways in Archaea

Glucose phosphorylation

As Archaea lack the bacterial PEP-dependent phosphotransferase system-like transport systems, the initial phosphorylation of glucose is the first activation step in the EM modifications. In Archaea, a great variety of enzymes, which differ in specificity and their phosphoryl donor, is observed. In Euryarchaeota, ADP-dependent glucokinases, which exhibit high specificity for glucose, were identified and characterized (e.g. in *P. furiosus* [5,23], *Thermococcus litoralis* [24] and *A. fulgidus* strain 7324 [25]). In addition, a bifunctional ADP-dependent glucokinase/phosphofructokinase, which is active on both glucose and fructose 6-phosphate, was described in

Figure 3



Modifications of the Entner–Doudoroff (ED) pathway in Archaea. **(a)** The non-phosphorylative ED pathway (enzymes 1–7) is operative in *Thermoplasma acidophilum*. This pathway is not coupled with a net ATP yield ('non-phosphorylative'). **(b)** The semi-phosphorylative ED pathway (enzymes 1, 2, 8, 3, 9–12, 6 and 7) is present in halophilic Archaea. Owing to the phosphorylative GAPDH/phosphoglycerate kinase enzyme couple, this ED modification yields one ATP. A branched ED (i.e. a combination of both non-phosphorylative [a] and semi-phosphorylative [b] ED pathways) is operative in *S. solfataricus* and *T. tenax*. Owing to non-phosphorylative GAPN, the net ATP yield is zero. Abbreviations: 1.3 BPG, 1,3-bisphosphoglycerate; Fd_{ox} and Fd_{red}, oxidized and reduced ferredoxin; GA, glyceraldehyde; GAP, glyceraldehyde-3 phosphate; KDG, 2-keto-3-deoxy-gluconate; KDPG, 2-keto-3-deoxy-6-phosphogluconate; PEP, phosphoenolpyruvate; 2 PG, 2-phosphoglycerate; 3 PG, 3-phosphoglycerate. Enzymes are nnumbered as follows: 1, glucose dehydrogenase; 2, gluconate dehydratase; 3, KD(P)G aldolase; 4, glyceraldehyde dehydrogenase (poposed for *T. acidophilum* [12•]), glyceraldehyde:ferredoxin oxidoreductase (proposed for *T. tenax* [76]) or glyceraldehyde oxidoreductase (proposed for *S. acidocaldarius* [101]); 5, glycerate kinase; 6, enolase; 7, pyruvate kinase; 8, KDG kinase; 9, GAPDH; 10, phosphoglycerate kinase; 11, GAPN; 12, phosphoglycerate mutase.

Methanococaldococcus jannaschii [26]. Structural analysis of the enzymes from *T. litoralis* [27] and *Pyrococcus horikoshii* [28] grouped the ADP-dependent kinases in the ribokinase superfamily (SCOP; structural classification of proteins). In Crenarchaeota (e.g. *Aeropyrum pernix* [29,30] and *T. tenax* [31]), ATP-dependent glucokinases with broad hexokinase-like substrate specificity (e.g. glucose, fructose, mannose and 2-deoxyglucose) were identified. The ATP–dependent enzymes are members of the ROK family (repressor protein, open reading frame, sugar kinase), which belong to the actin-ATPase superfamily (SCOP). No archaeal glucokinases exhibit allosteric properties and thus differ from eukaryotic hexokinases, which constitute an allosteric control step in the classical EM pathway.

Glucose 6-phosphate/fructose 6-phosphate isomerization

The isomerization of glucose 6-phosphate in Archaea is catalyzed by three different protein families. Homologs of the classical phosphoglucose isomerase (PGI) superfamily, which comprise almost all PGIs in Eukarya and Bacteria, were identified in only three Archaea: in two halophiles (*Haloarcula marismortui* and *Halobacterium* NRC1) and in *M. jannaschii*. The enzyme from *M. jannaschii* was characterized [32].

In the Euryarchaeota *P. furiosus*, *Thermococcus litoralis*, *A. fulgidus* and *Methansosarcina mazei*, a novel type of metal-dependent PGIs have been identified and characterized [33,34,35•,36]. These PGIs belong to the cupin super-

family (cPGI) and thus represent a convergent line of PGI evolution. The crystal structure of a cPGI from *P. furiosus* revealed a typical cupin fold [37–39], and a hydride mechanism of glucose 6-phosphate isomerization was proposed [37] In *A. pernix*, *P. aerophilum*, *T. acidophilum* [40,41] and *T. tenax* [12•] an unusual PGI was described that differs from all knows PGIs; this catalyzes the isomerization of both glucose-6-phosphate and mannose-6-phosphate at similar catalytic efficiency, defining the enzyme as bifunctional phosphoglucose/phosphomannose isomerase (PGI/PMI). PGI/PMIs represent a novel family within the PGI superfamily, as proven by the crystal structure of the *P. aerophilum* enzyme [42]; a structural basis for bifunctionality was proposed [43].

Fructose 6-phosphate phosphorylation

The phosphorylation of fructose-6-phosphate is catalyzed by different enzymes that vary in respect to their phosphoryl donor PP_i, ADP and ATP. Whereas the PP_i-dependent phosphofructokinase is reversible (*T. tenax* [12•,44]), the ADP-dependent phosphofructokinases (in *P. furiosus* [5,45], *Thermococcus zilligii* [46], *A. fulgidus* 7324 [47] and glycogen-forming methanogenic Archaea [48]) and the ATP-dependent 6-phophofructokinases (in *Desulfurococcus amylolyticus* [49] and *A. pernix* [50,51]) represent unidirectional glycolytic enzymes.

Phylogenetic analysis revealed that the PP_i-dependent phophofructokinase is related to classical ATP-dependent enzymes and is a member of the phosphofructokinase A (PFK A) family [44]. ADP-dependent phosphofructokinases belong to the ribokinase superfamily [27]. The archaeal ATP-dependent phosphofructokinases are members of PFK B family, which also belong to the ribokinase superfamily. The crystal structure of an archaeal PFK B homolog, an ATP-dependent nucleoside kinase from *Methanocaldococcus jannaschi*, was solved [52].

Strikingly, for all archaeal phosphofructokinases described to date, no allosteric regulation by classical effectors of bacterial and eukaryal ATP-dependent phosphofructokinases was observed. This indicates that these enzymes do not represent a site of allosteric control in archaeal glycolysis.

Fructose 1,6-bisphosphate cleavage

FBP aldolase catalyzes the reversible cleavage of FBP to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. Archaeal FBP aldolases show no obvious sequence similarity to the classical bacterial and eukaryal class I and II FBP aldolases, but form a new family of archaeal-type class I FBP aldolases (in *T. tenax* and *P. furiosus* [53]). Structural analysis of the *T. tenax* enzyme revealed new insights in the evolution of $(\beta\alpha)_8$-barrel proteins [54] and the reaction mechanism of Schiff base forming FBP aldolases [55]. Isomerization of dihydroxyacetone phosphate to glyceraldehydes 3-phosphate is catalyzed by triosephosphate isomerases (see [56]).

Glyceraldehyde 3-phosphate oxidation

In Archaea, the oxidation of GAP in glucose degradation is catalyzed by either glyceraldehyde-3-phosphate:ferredoxin oxidoreductase (GAPOR; in *P. furiosus* [57,58], *A. fulgidus* strain 7324 and *Pyrobaculum aerophilum*; Reher *et al.*, personal communication) and/or non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase (GAPN; in *T. tenax* [59] and *S. solfataricus*; [4•], Ettema *et al.* personal communication). Both enzymes catalyze the irreversible, non-phosphorylating oxidation of GAP to 3-phosphoglycerate using either ferredoxin or $NAD(P)^+$ as electron acceptors. GAPOR belongs to the aldehyde ferredoxin oxidoreductase superfamily. The archaeal GAPN is a member of the aldehyde dehydrogenase superfamily, but the characterized enzymes of *T. tenax* and *S. solfataricus* are unusual in respect to their allosteric properties ([4•,59] Ettema *et al.* personal communication). The *T. tenax* enzyme is controlled by the energy charge of the cell and by early intermediates of the EM pathway as well as by glycogen metabolism, suggesting a central role in the regulation of glycolysis. The crystal structure of the *T. tenax* GAPN was solved [60]. The structural basis for the allosteric regulation of the *T. tenax* enzyme was deciphered and based on sequence comparisons allosteric properties were predicted for the enzymes of *Sulfolobus tokodaii*, *S. solfataricus*, *A. pernix* and *P. furiosus* [61].

All Archaea contain the classical type of glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which catalyzes the reversible phosphorylating oxidation of GAP to yield 1,3-bisphosphoglycerate. In Archaea, with the exception of glycolysis in halophilic species (Figure 3), GAPDH appears to be exclusively involved in gluconeogenesis. Enzymatic as well as transcript analyses in *P. furiosus* [58,62,63] and *T. tenax* [4•,12•,64], which harbour all three GAP-converting enzymes, suggest a catabolic function for GAPN and GAPOR and an anabolic function for the GAPDH/phosphoglycerate kinase enzyme couple.

3-Phosphoglycerate/2-phosphoglycerate interconversion

Phosphoglycerate mutase (PGM) catalyzes the interconversion of 3-phosphoglycerate and 2-phosphoglycerate. Two distinct PGM types have been described that differ in their requirement for 2,3-bisphosphoglycerate (dPGM) as the phosphoryl donor. In Archaea, distant homologues of cofactor-independent PGM (iPGM) and dPGM have been identified. iPGM, which belongs to the alkaline phosphatase (binuclear metalloenzyme) superfamily, was characterized (in *P. furiosus*, *M. jannaschii* [65,66] and *S. solfataricus* [67]).

Phosphoenolpyruvate conversion to pyruvate

Enolase catalyses the formation of phosphoenolpyruvate from 2-phosphoglycerate; this enzyme has been characterized in *P. furiosus* [68]. The conversion of phosphoenolpyruvate to pyruvate in Archaea is catalyzed by two

different enzymes. Pyruvate kinase (PK; *T. tenax* [69], *A. fulgidus*, *A. pernix* and *P. aerophilum* [70]) catalyzes the irreversible energy-yielding formation of pyruvate. In contrast to bacterial and eukaryal PKs, these archaeal PKs exhibit reduced regulatory potential, and thus another important allosteric control point present in the classical EM pathway appears to be absent in archaeal modifications. However, an AMP-stimulated PK was described in *T. acidophilum* [71]. Pyruvate phosphate dikinase in *T. tenax* catalyzes the reversible interconversion of PEP and pyruvate ($ATP + P_i + pyruvate \leftrightarrow AMP + PP_i + PEP$) (Tjaden *et al.*, personal communication; [12•]).

Pyruvate conversion to acetyl-CoA

All Archaea, both anaerobes and aerobes, convert pyruvate to acetyl CoA by way of pyruvate:ferredoxin oxidoreductase (see in [17]). A functional pyruvate dehydrogenase complex, typical for Eukarya and Bacteria, has not been described in Archaea to date, although homologous genes were found in *T. acidophilum* and in halophilic Archaea [72,73].

Acetyl-CoA conversion to acetate

Several anaerobic fermentative hyperthermophilic Archaea and aerobic halophiles [74] generate significant amounts of acetate as a product of glucose fermentation (Figure 1). It was found that in these Archaea, the formation of acetate from acetyl CoA is catalyzed by a novel prokaryotic enzyme, acetyl-CoA-synthetase (ADP-forming) (ACD) ($acetyl\text{-}CoA + ADP + P_i \bullet acetate + ATP + CoA$) [75]. This represents the major energy-conserving reaction in anaerobic sugar-fermenting hyperthermophiles. By contrast, all Bacteria, including the hyperthermophile *Thermotoga*, convert acetyl-CoA to acetate by way of two enzymes: phosphotransacetylase and acetate kinase [19]. ACDs from several hyperthermophiles and from *Haloarcula marismortui* have been characterized [76–81].

Modified Entner–Doudoroff pathways in Archaea

Initial studies in Archaea revealed the presence of the non-phosphorylative ED pathway, which involves 2-keto-3-deoxy gluconate (KDG) cleavage, glyceraldehyde oxidation and glycerate phosphorylation in the (hyper)thermophilic Archaea (*S. solfataricus* [2], *T. acidophilum* [1] and *T. tenax* [6,9–11]). The semi-phosphorylative ED pathway, which involves KDG kinase, KDPG cleavage and GAP oxidation by GAPDH, was identified as a catabolic route for glucose in halophiles [13,14]. However, a more recent comparative genomics-based approach indicates the presence of a ‘branched’ (i.e. a non- and semi-phosphorylative) ED modification that involves KDG kinase, a bifunctional KD(P)G aldolase and GAPN in the hyperthermophiles *S. solfataricus* and *T. tenax* [4•,12•]. The non-phosphorylative ED pathway was recently shown to be promiscuous and represents an equivalent route for glucose and galactose catabolism in *S. solfataricus* [3,18•], whereas the pathway seems to be specific for glucose in *T. tenax* [4•] (Figure 3).

Glucose oxidation

Glucose dehydrogenase catalyzes the oxidation of glucose to yield gluconate (in *T. acidophilum* [82], *Haloferax mediterranei* [83], *T. tenax* [10], *S. solfataricus* [3,84,85] and *Picrophilus torridus* [86]). According to pathway promiscuity, the enzymes of *S. solfataricus*, *P. torridus* and *T. acidophilum* show high activities when using galactose as a substrate, whereas no significant activity with galactose was observed for the *T. tenax* enzyme. The structures of the glucose dehydrogenases of *T. acidophilum* [87] and of *H. mediterranei* [88] have been solved.

Gluconate dehydration

Gluconate dehydratase catalyzes the dehydration of the respective sugar acid. For the enzyme from *S. solfataricus* promiscuity for both gluconate and galactonate was reported by Lamble *et al.* [89] but Kim and Lee did not find that this promiscuity occurred [90]; the *T. tenax* enzyme was shown to be specific for gluconate [4•]. The enzyme is a member of the enolase superfamily and shows no similarity to the classical ED dehydratase [4•,89,90].

2-keto-3-deoxy gluconate/2-keto-3-deoxy-(6-phospho)-gluconate cleavage

KDG and KDPG generated in ED modifications of both *S. solfataricus* and *T. tenax* are cleaved by bifunctional KD(P)G aldolase [4•]. This enzyme catalyzes the reversible aldol cleavage of non-phosphorylated substrates (e.g. KDG and glyceraldehyde) [91] as well as of phosphorylated substrates (e.g. KDPG and glyceraldehyde 3-phosphate) [4•]. Furthermore, lack of facial selectivity was demonstrated for the *S. solfataricus* enzyme in catalyzing the cleavage of KDG as well as of 2-keto-3-deoxygalactonate (KDgal), both of which yield glyceraldehydes and pyruvate [3]. The enzyme is a member of the *N*-acetyl-neuraminate lyase superfamily and shows no similarity to the classical ED aldolase [4•,91]. The crystal structure of the *S. solfataricus* KD(P)G aldolase was resolved and binding sites for the non-phosphorylated substrates KDG, KDGal and glyceraldehyde were determined, providing a structural basis for the promiscuity of these substrates [92].

2-keto-3-deoxy gluconate phosphorylation

KDG kinase is a member of the ribokinase superfamily and catalyzes the ATP-dependent phosphorylation of KDG to yield KDPG. KDG kinase represents the key enzyme in the semi-phosphorylative ED modifications in halophilic Archaea and in *T. tenax* and *S. solfataricus* [4•].

Energetics of modified Embden–Meyerhof and Entner–Doudoroff pathways

The net ATP yields of the classical EM and ED pathway are 2 mol and 1 mol ATP per mol glucose, respectively.

The formal net ATP yield of the modified EM pathways that involve non-phosphorylative GAPOR or GAPN is zero (or <1 ATP in *T. tenax*, assuming that the anabolic-formed PP_i, a waste product of the cell, is recycled by PP_i-dependent PFK; Figure 2). For *Pyrococcus*, an additional site of ATP formation has been proposed to occur by way of electron transport phosphorylation coupled to H_2 formation via ferredoxin-dependent hydrogenase [93] or via reversal of PEP synthetase [22]. Formally, the net ATP yields of non-phosphorylative ED (as proposed for *T. acidophilum* [4•]) and of the branched ED variants (non- and semi-phosphorylative) of *T. tenax* and *S. solfataricus* that involve non-phosphorylative GAPN are zero. The semi-phosphorylative ED pathway of halophilic Archaea, which involves phosphorylating GAPDH and phosphoglycerate kinase, yields one ATP (Figure 3).

Gluconeogenesis in Archaea

Gluconeogenesis (i.e. glucose 6-phosphate formation from pyruvate) proceeds in lithoautotrophic (e.g. methanogens) and organonotrophic Archaea, as in Bacteria and Eukarya, by way of the reversible reactions of the EM pathway. Accordingly, the irreversible reactions of the modified EM pathway in Archaea (i.e. ADP- and ATP-dependent PFKs, GAPOR and GAPN, and PKs) are reversed by different enzymes: fructose-1,6-bisphosphatase (FBPase), classical GAPDH/phosphoglycerate kinase and phosphoenolpyruvate synthetases (PEPS), respectively.

Enzymatic and mutational analysis in *Thermococcus kodakaraensis* revealed that the FBPase class V represents the functional FBPase in Archaea [21,94•]. In *T. tenax*, reversible PP_i-dependent PFK substitutes for FBPase in gluconeogenesis.

The formation of GAP from 3-phosphoglycerate in Archaea in the course of gluconeogenesis, which counteracts the irreversible GAPOR and GAPN reactions, is catalyzed by the $NADP^+$-dependent GAPDH and the phosphoglycerate kinase enzyme couple (for references see in [20]). Both enzymes are kinetically regulated in the gluconeogenetic direction (as shown for *P. furiosus* [62] and *T. tenax* [64]). In halophilic Archaea, GAPDH and phosphoglycerate kinase have a glycolytic function in the semi-phosphorylative ED pathway. The structure of GAPDH from *S. solfataricus* [95] and from *M. fervidus* [96] has been solved.

PEPS counteracts the PK reaction. PEPS of *T. tenax* is a true anabolic enzyme (Tjaden *et al.*, personal communication, [12•]), whereas a function in both glycolysis and gluconeogenesis was proposed for PEPS of *P. furiosus* [97,98].

Conclusions

The current analysis of archaeal sugar metabolism and the characterization of the enzymes involved revealed several unusual pathways that are significantly different to the classical EM and ED pathways. In particular, differences in sugar-phosphorylating and -isomerizing enzymes and in GAP-oxidizing enzymes were found. The biochemical, phylogenetic and structural analysis of these novel archaeal enzymes contributes, for example, to the understanding of the complexity of enzyme superfamilies.

Increasing archaeal genome sequence information, and thus comparative and functional genomic approaches [4•,12•,20,21,63], allowed predictions with respect to reconstruction of archaeal carbohydrate metabolism and — together with classical biochemical methods — the identification of the unusual glycolytic pathways and enzymes. Several novel enzymes (e.g sugar isomerases) and their encoding genes were identified after initial biochemical characterization.

Although the routes of hexose catabolism have been unraveled in several Archaea, the regulation as well as energetics of the archaeal glycolytic pathways are still not well understood. In view of the apparent absence of classical allosteric sites, a primary regulation of glycolytic fluxes in Archaea might proceed at the level of gene expression [21]. Current analyses hint that there might be control points at the level of glyceraldehyde-3-phosphate, which also includes allosteric control of GAPN. Furthermore, to date, the pathways of pentose degradation [99] and of hexose/pentose conversions have not been studied in detail in Archaea [100].

Clearly, future functional genomics studies combined with biochemical characterization of enzymes will provide further new insights and will extend our current understanding of central carbohydrate metabolism and its regulation in Archaea.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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