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ORIGINAL PAPER

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Sugar utilization in the hyperthermophilic, sulfate-reducing archaeon *Archaeoglobus fulgidus* strain 7324: starch degradation to acetate and CO_2 via a modified Embden-Meyerhof pathway and acetyl-CoA synthetase (ADP-forming)

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Abstract The hyperthermophilic, sulfate-reducing archaeon *Archaeoglobus fulgidus* strain 7324, rather than the type strain VC16, was found to grow on starch and sulfate as energy and carbon source. Fermentation products and enzyme activities were determined in starch-grown cells and compared to those of cells grown on lactate and sulfate. During exponential growth on starch, 1 mol of glucose-equivalent was incompletely oxidized with sulfate to approximately 2 mol acetate, 2 mol CO_2 and 1 mol H_2S. Starch-grown cells did not contain measurable amounts of the deazaflavin factor F_{420} (<0.03 nmol/mg protein) and thus did not show the F_{420}-specific green-blue fluorescence. In contrast, lactate (1 mol) was completely oxidized with sulfate to 3 mol CO_2 by strain 7324, and lactate-grown cells contained high amounts of F_{420} (0.6 nmol/mg protein). In extracts of starch-grown cells, the following enzymes of a modified Embden-Meyerhof pathway were detected: ADP-dependent hexokinase (ADP-HK), phosphoglucose isomerase, ADP-dependent 6-phosphofructokinase (ADP-PFK), fructose-1,6-phosphate aldolase, glyceraldehyde-3-phosphate:ferredoxin oxidoreductase (GAP:FdOR), phosphoglycerate mutase, enolase, and pyruvate kinase (PK). Specific activities of ADP-HK, ADP-PFK, GAP:FdOR, and PK were significantly higher in starch-grown cells than in lactate-grown cells, indicating induction of these enzymes during starch catabolism. Pyruvate conversion to acetate involved pyruvate:ferredoxin oxidoreductase and ADP-forming acetyl-CoA synthetase. The findings indicate that the archaeal sulfate reducer *A. fulgidus* strain 7324 converts starch to acetate via a modified Embden-Meyerhof pathway and acetyl-CoA synthetase (ADP-forming). This is the first report of growth of a sulfate reducer on starch, i.e. on a polymeric sugar.

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Introduction

Hyperthermophilic prokaryotes, with an optimal growth temperature higher than 80 °C (Stetter 1996), are considered to represent the phylogenetic most ancestral organisms (Stetter 1996, 1999). Various hyperthermophiles, including the archaea *Sulfolobus*, *Pyrococcus*, *Thermococcus*, *Thermoproteus*, *Desulfurococcus*, and the bacterium *Thermotoga*, have been shown to grow on sugars such as starch, glucose, maltose, and cellobiose (see Stetter 1996; Schönheit and Schäfer 1995; de Vos et al. 1998; Danson 1993). Comparative analyses of the sugar degradation pathways in these hyperthermophiles indicate that the classical Embden-Meyerhof or Entner-Doudoroff pathway is operative only in the bacterium *Thermotoga*, whereas all archaea degrade sugars via modified versions of these pathways. All anaerobic species (*Pyrococcus*, *Thermococcus*, *Thermoproteus*, *Desulfurococcus*) degrade sugars predominanly via modified Embden-Meyerhof pathways, in which novel kinases, such as ADP-dependent hexokinase and ADP-dependent 6-phosphofructokinase, and unusual enzymes of glyceraldehyde 3-phosphate oxidation, such as GAP:FdOR or non-phosphorylating, NAD^+-reducing glyceraldehyde-3-phosphate dehydrogenase are operative (for references see Schönheit and Schäfer 1995; Selig et al. 1997; de Vos et al. 1998; Brunner et al. 1998; Hansen and Schönheit 2000).

To analyze sugar degradation pathways in archaea further, we were interested in the sugar metabolism of *Archaeoglobus fulgidus*, which represents the first isolated sulfate reducer in the domain of Archaea. The type strain, *A. fulgidus* VC16, was isolated by Stetter (Stetter et al.

1987; Stetter 1988) from hydrothermal areas near Volcano (Italy). With a temperature optimum of 83 °C, it belongs to the group of hyperthermophiles (Stetter 1996, 1999). Later, *A. fulgidus* strain Z (Zellner et al. 1989a) and strain 7324 (Beeder et al. 1994) were isolated from Vulcano and from hot oil-field waters of the North Sea (Norway), respectively. *A. fulgidus* belongs to the euryarchaeotal branch of the Archaea, being closely related to methanogens and more distantly related to the *Thermococcales* (see Thauer and Kunow 1995).

Most metabolic studies have been performed with *A. fulgidus* strain VC16 (see Thauer and Kunow 1995); recently, the complete genome of this organism has been sequenced (Klenk et al. 1997). *A. fulgidus* VC16 grows chemolithoautotrophically on H_2 and CO_2, with sulfate or thiosulfate as electron acceptor. Preferentially, it grows chemoorganoheterotrophically with sulfate and lactate, but also on pyruvate, formate, peptone and yeast extract as electron donors. Lactate was found to be completely oxidized to 3 CO_2 by *A. fulgidus* VC16. The pathway of lactate oxidation – via acetyl-CoA – has been elucidated (see Thauer and Kunow 1995). Acetyl-CoA oxidation proceeds via a modified oxidative acetyl-CoA/carbon monoxide dehydrogenase pathway involving cofactors (5-deazaflavin F_{420}, methanopterin, methanofuran) and enzymes previously thought to be specific for methanogenic archaea (Thauer and Kunow 1995; Möller-Zinkhan et al. 1989). Due to the presence of the deazaflavin factor F_{420}, *Archaeoglobus* species show an intensive blue-green fluorescence at 420 nm under the epifluorescence microscope.

The utilization of sugars by *A. fulgidus* species has not unequivocally been demonstrated so far. *A. fulgidus* VC 16 has been reported to grow on starch and glucose in minimal medium; however, growth characteristics and fermentation balances on sugars have not been presented (Stetter et al. 1987). In a later study, growth of *A. fulgidus* strain VC16 and strain Z on glucose in the presence of yeast extract and peptone could not be shown (Zellner et al. 1989a). Furthermore, in the complete sequenced genome of *A fulgidus* VC16, conserved genes of key enzymes of sugar degradation pathways, e.g. hexokinase, 6-phosphofructokinase, and pyruvate kinase, in the case of the classical Embden-Meyerhof pathway, could not be identified (Cordwell 1999). Also, genes showing similarity to the encoding genes of the newly recognized ADP-dependent hexokinase and ADP-dependent phosphofructokinase in the modified Embden-Meyerhof pathway of the hyperthermophilic archaeon *Pyrococcus furiosus* (Tuininga et al. 1999) were not found in the *A. fulgidus* VC 16 genome. Thus, utilization of sugars as energy substrates by *A. fulgidus* species was doubted (see Zellner et al. 1989a; Klenk et al. 1997; de Vos et al. 1998; Cordwell 1999).

In this communication, we investigated the utilization of sugars in the *A. fulgidus* strains VC 16 and 7328. It was found that *A. fulgidus* strain 7327, rather than strain VC16, can grow on starch and sulfate as carbon and energy source. Fermentation balances showed that starch was incompletely oxidized to acetate and CO_2. Enzyme studies indicate that starch degradation to pyruvate proceeds via a modified Embden-Meyerhof pathway and that acetate formation involves ADP-forming acetyl-CoA synthetase.

Materials and methods

Growth of *Archaeoglobus fulgidus* strains VC16 and 7324

Both organisms were grown anaerobically at 76 °C in closed bottles on media described by Möller-Zinkhan et al. (1989) with modifications. The basal medium contained (per 1 l) 100 ml salt solution and 10 ml trace element solution (for composition see below), 5.5 g $NaHCO_3$, 2 mg $(NH_4)_2Fe(SO_2)_2 \cdot 6H_2O$, 0.5 g yeast extract, and, as indicated, 10 mmol L-lactate or various sugars, including starch, as carbon and energy source. The salt solution contained (per 1 l) 74 g $MgSO_4 \cdot 7H_2O$, 3.4 g KCl, 27.5 g $MgCl_2 \cdot 6H_2O$, 2.5 g NH_4Cl, 1.4 g $CaCl_2 \cdot 2H_2O$, 1.4 g K_2HPO_4, 180 g NaCl, 5 ml resazurin (0.2% w/v). The trace element solution contained (per 1 l) 1.5 g Titriplex I, 3 g $MgSO_4 \cdot 7H_2O$, 0.01 g Na_2WO_4, 0.01 g H_3BO_3, 0.01 g $Na_2MoO_4 \cdot 2H_2O$, 0.025 g $NiCl_2 \cdot 6H_2O$, 0.3 g $Na_2SeO_3 \cdot 5H_2O$, 0.45 g $MnSO_4 \cdot 1H_2O$, 1.0 g NaCl, 0.1 g $FeSO_4 \cdot 7H_2O$, 0.18 g $CoSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.18 g $ZnSO_4 \cdot 7H_2O$, 0.01 g $CuSO_4 \cdot 5H_2O$, 0.02 g $KAl(SO_4)_2 \cdot 12H_2O$. The pH was adjusted to 6.5 with KOH.

The medium was adjusted to pH 6.8 with HCl, evacuated and gassed with N_2:CO_2 (80:20, v/v). After heat sterilization, the medium was reduced by the addition of 0.5 ml 0.5 M Na_2S and 0.5 ml 0.5 M $Na_2S_2O_4$ per 50 ml. The medium was inoculated with 2–5% exponential phase cells and growth was followed by the increase in the total cell number using a Helber counting chamber. The protein content of cells was measured using a modified Biuret method (Bode et al. 1968). A cell number of 1×10^8 cells/ml corresponded to a protein content of about 0.07 mg/ml. Large amounts of cell mass of *A. fulgidus* 7324 were obtained after growth on starch and sulfate or lactate and sulfate in a 100-l Biostat fermenter from Braun (Melsungen, Germany).

Growth experiments

Various sugars (10 mM each of maltose, glucose, and fructose, or 1 g/l each of sucrose, cellobiose and starch) were tested as substrates in the presence of 0.05% yeast extract and sulfate (30 mM). Growth was followed by counting cell numbers for three to six transfers (each 2% inoculum) on the medium containing the particular sugar. The fermentation balances in growing cultures of *A. fulgidus* strain 7324 were determined in 125-ml serum bottles filled with 50 ml medium containing sulfate and either lactate or starch as substrates. In these experiments, the media did not contain $NaHCO_3$ and the gas phase was 100% N_2 (0.5 bar). At the times indicated in Fig. 1A, B, samples were removed and analyzed for L-lactate, starch, acetate and CO_2. H_2S was determined in separate cultures after trapping H_2S from the gas phase into the medium by addition of NaOH (final pH 11) (Selig and Schönheit 1994). The resulting H_2S concentration in the medium represents the total amount of H_2S formed during growth and was assayed colorimetrically (Cline 1969). The fermentation balances were corrected for growth and product formation on yeast extract (0.05%) in the absence of lactate and starch, respectively.

Preparation of cell extracts

Cell extracts were prepared under oxic conditions. Cells were harvested in the late exponential phase, cooled to 4 °C and centrifuged for 20 min at 10,000×*g* at 4 °C. The cells were resuspended in 100 mM Tris/HCl, pH 8.0, or 50 mM potassium phosphate buffer, pH 7.0, respectively, and stored at –20 °C. For preparing cell-free extracts the suspension was passed three times through a French

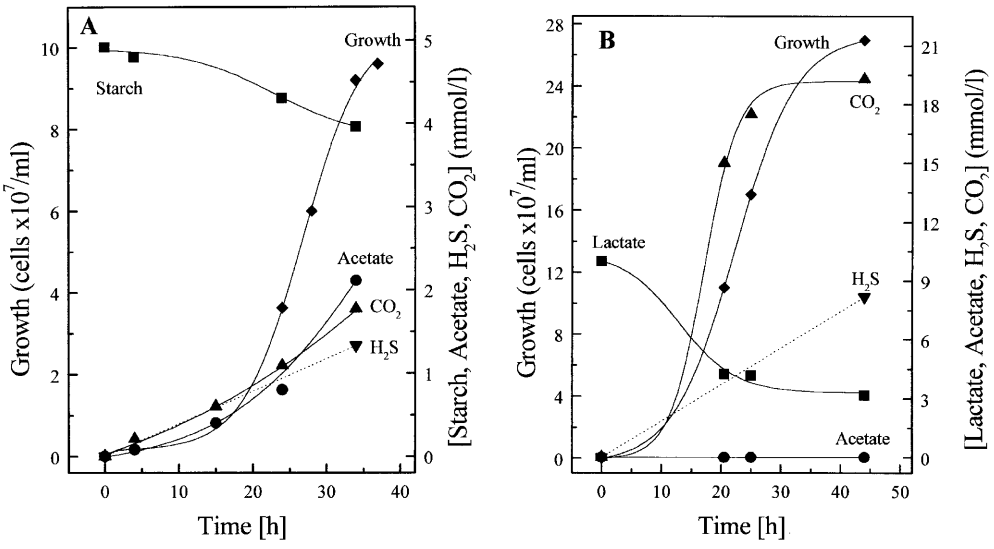

Fig. 1 A, B Starch degradation to **A** CO_2,acetate, and H_2S and **B** lactate degradation to CO_2 and H_2S in growing cultures of *Archaeoglobus fulgidus* strain 7324. The organism was grown at 76 °C in 125-ml closed serum bottles filled with 50 ml medium containing 0.05% yeast extract and 1 g starch/l or 10 mM lactate. Growth (cell number, ◆), the consumption of lactate or starch [given as glucose-equivalents] (■), and the formation of CO_2 (▲), H_2S (▼), and acetate (●) were determined at the times indicated

pressure cell at 1×10^7 Pa and centrifuged (15 min at 10,000×*g*, 4 °C). Protein was determined by the method of Bradford (1976) using bovine serum albumin as standard.

Determination of enzyme activities

Enzyme assays were carried out aerobically at 50 or 80 °C in cuvettes filled with 1 ml of assay mixture. Enzyme assays following benzylviologen reduction were carried out under anoxic conditions in stoppered glass cuvettes with N_2 (0.2 bar) as gas phase. Coupled enzyme assays containing auxiliary enzymes were performed between 50 and 60 °C. The auxiliary enzymes were generally added shortly before the start of the reaction and it was ensured that these enzymes were not rate-limiting. One unit (1 U) of enzyme activity is defined as 1 µmol substrate consumed or product formed per min. In case of viologen dye reduction, 1 U is equal to 2 µmol viologen dye reduced per min. Assay mixtures containing viologen dyes were slightly reduced by the addition of a sodium dithionite solution prior to the start of the reaction.

Hexokinase activity, dependent either on ADP, ATP or pyrophosphate (PP_i), was determined at 50 °C by measuring the ADP-, ATP- or PP_i-dependent conversion of glucose to glucose 6-phosphate coupled with the reduction of $NADP^+$ via glucose-6-phosphate dehydrogenase. The assay mixture contained 100 mM Tris/HCl, pH 8.0, 10 mM glucose, 2 mM each of ADP, ATP or PP_i, 10 mM $MgCl_2$, 1 mM $NADP^+$, 1 U glucose-6-phosphate dehydrogenase, and extract.

Phosphofructokinase activity, dependent either on ADP, ATP or PP_i, was determined at 50 °C by coupling the ADP-, ATP- or PP_i-dependent formation of fructose 1,6-phosphate from fructose 6-phosphate with the oxidation of NADH using FBP aldolase, triosephosphate isomerase, and glycerol-3-phosphate dehydrogenase. The assay contained 100 mM Tris/HCl, pH 8.0, 5 mM fructose 6-phosphate, 2 mM of each ADP, ATP or PP_i, 10 mM $MgCl_2$, 0.3 mM NADH, 3 U fructose-1,6-phosphate aldolase, 4 U triosephosphate isomerase, 1 U glycerol-3-phosphate dehydrogenase, and extract.

Pyruvate kinase activity was determined at 50 °C by coupling the ADP-dependent formation of pyruvate and ATP with the oxidation of NADH by lactate dehydrogenase. The assay mixture contained 100 mM Tris/HCl, pH 8.0, 5 mM phosphoenolpyruvate, 2 mM ADP, 10 mM $MgCl_2$, 0.3 mM NADH, 15 U lactate dehydrogenase, and extract.

Glyceraldehyde 3-phosphate:ferredoxin oxidoreductase (GAP:FdOR) activity was determined at 50 °C by following GAP-dependent reduction of benzylviologen, as ferredoxin substitute, at 578 nm (see in Selig et al. 1997). GAP was generated from FBP via FBP aldolase. The assay contained 100 mM Tris/HCl, pH 8.0, 5 mM FBP, 5 mM benzylviologen, 1 U FBP aldolase, and extract. For determination of the K_m value, the assay contained 100 mM Tris/HCl, pH 8.0, 0.03–0.6 mM GAP, 5 mM benzylviologen, and extract.

Other enzyme activities

Glucose-6-phosphate isomerase, fructose-1,6-bisphosphate aldolase, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, 3-phosphoglycerate kinase, phosphoglycerate mutase, enolase, pyruvate kinase, pyruvate:ferredoxin oxidoreductase, acetyl-CoA synthetase (ADP-forming), malate dehydrogenase, ATPase, carbon monoxide dehydrogenase, acetate kinase, phosphate acetyltransferase, adenylate kinase, and NADP:ferredoxin oxidoreductase were measured as described previously (Schäfer and Schönheit 1991, 1992; Schäfer et al. 1993; Schröder et al. 1994).

Gel filtration of cell extracts

Hexokinase activity and phosphofructokinase activity in crude extracts of starch-grown cells of *A. fulgidus* 7324 were separated from ATPase activity by gel filtration using a FPLC system from Pharmacia Biotech (Freiburg, Germany). Cell free extractswere centrifuged (15 min, 10,000×*g*) and the supernatant was filtered through a 0.2-µm-pore-size filter (Sartorius AG, Göttingen, Germany). The resulting extract, containing 5.4 mg protein, was applied to a gel filtration column (Superdex TM^{200} 16/60) equilibrated with 50 mM Tris/HCl, pH 8.0, 150 mM NaCl. Proteins were eluted with the same buffer at a flow rate of 1 ml/min. Fractions (1 ml) were collected and analyzed for activities of hexokinase, phosphofructokinase, and ATPase. ADP-dependent hexokinase activity eluted between 67 and 77 ml; ADP-dependent 6-phosphofructokinase eluted between 60 and 67 ml. In these fractions ATPase activity was not found.

Analytical procedures

CO_2 and acetate were determined by gas chromatography as described by Selig et al. (1997); starch and lactate were determined enzymatically (Beutler 1988; Noll 1988). Coenzyme factor F_{420} was isolated and quantified from 0.5 g wet weight cells, grown either on starch/sulfate or on lactate/sulfate, as described by Schönheit et al. (1981).

Materials

Yeast extract was from Difco (Stuttgart, Germany). Tris was from Biomol (Hamburg, Germany). 2,3-Bisphosphoglycerate, dihydroxyacetone phosphate, gluconate, glycerate, glyceraldehyde, glyceraldehyde 3-phosphate, and 3-phosphoglycerate were from Sigma (Deisenhofen, Germany). Enzymes and coenzymes were from Boehringer (Mannheim, Germany). If not mentioned otherwise, all other chemicals were reagent grade and obtained from E. Merck (Darmstadt, Germany) or Serva (Heidelberg, Germany). Gases (N_2, 5.0; CO; N_2/CO_2, 80%/20%) were from Linde (Hamburg, Germany). *Archaeoglobus fulgidus* strain VC 16 (DSM 4304) and *Archaeoglobus fulgidus* strain 7324 (DSM 8774) were from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany).

Results

Growth of *A. fulgidus* strain 7324 on starch and sulfate

Archaeoglobus fulgidus strains 7324 and strain VC 16 were tested with respect to their ability to grow on sugars and sulfate as carbon and energy source. Both *Archaeoglobus* strains, previously grown on lactate and sulfate, were incubated on media containing 0.5 g yeast extract/l, various sugars (glucose, fructose, maltose, sucrose, cellobiose, starch), and sulfate (see Methods section). After three to four transfers (each with 2% inoculum) on sugar-containing media, significant growth was observed only with *A.fulgidus* strain 7324 on starch and sulfate. The maximal cell densities reached were $1–2\times10^8$ cells/ml. No significant growth ($2–3\times10^7$ cells/ml) was observed with all other sugars tested. The observed low maximal cell densities were similar to those obtained in the absence of sugars due to growth on yeast extract (0.05%) present in the media. Growth of *A. fulgidus* 7324 on starch was dependent on sulfate and on low concentrations of yeast extract. No growth was observed either on starch/yeast extract in the absence of added sulfate (after three to four transfers with 2% inoculum) or on starch/sulfate in the absence of yeast extract. Routinely, 30 mM sulfate and 0.05% yeast extract were included in the growth medium.

A typical growth curve of *A. fulgidus* 7324 on starch and sulfate is shown in Fig. 1A. The organism grew exponentially with a doubling time of about 4–5 h up to 1×10^8 cells /ml. During the exponential growth phase, approximately 1 mM glucose-equivalent was converted to acetate, CO_2, and H_2S. Per mol of glucose-equivalent oxidized with sulfate approximately 2 mol acetate, 2 mol CO_2, and 1 mol H_2S were formed. For comparison, growth of *A. fulgidus* 7324 on lactate (10 mM) and sulfate (30 mM) was analyzed; the organism grew on these substrates with a doubling time of 4–5 h up to cell densities of about 3×10^8 cells/ml. Per mol of lactate consumed 3 mol CO_2 and 1.5 mol H_2S were formed, indicating that lactate, in contrast to starch, was completely oxidized to CO_2 by *A. fulgidus* strain 7324 (Fig. 1B).

For *A. fulgidus* strain VC16, significant growth on all sugars tested, including starch, could not be demonstrated; after three to four transfers (each 2% inoculum) on the particular sugars, the maximal cell densities ($2–5\times10^7$ cells/ml) were similar to those obtained on yeast extract alone. The findings indicate that *A. fulgidus* strain 7324, rather than strain VC 16, is able to grow on starch and sulfate as carbon and energy source under the growth conditions described.

Coenzyme F_{420} content of *Archaeoglobus fulgidus* strain 7324

Starch-grown cells of *A. fulgidus* strain 7324 were similar to lactate-grown cells with respect to their cell form and size. However, starch-grown cells did not show the green-blue deazaflavine-F_{420}-specific fluorescence under the epifluoromicroscope, whereas lactate-grown cells did show the intensive F_{420}-caused fluorescence (see Beeder et al. 1994) observed in all *Archaeoglobus* species. Upon transfer of *A. fulgidus* 7324 from lactate/sulfate medium to starch/sulfate medium, the F_{420}-specific fluorescence decreased during growth. Conversely, F_{420}-specific fluorescence resumed during growth on lactate/sulfate medium after transfer from starch/sulfate medium.

The amount of the deazaflavine F_{420} was quantified in *A fulgidus* 7324. In accordance with the microscopic observation, the F_{420} content in starch-grown cells was almost not measurable (<0.03 nmol/mg cell protein) whereas lactate-grown cells contained high amounts of F_{420} (0.6 nmol/mg protein). Similar F_{420} amounts have been reported for lactate-grown *A. fulgidus* VC 16 (Vorholt et al. 1995). The data suggest that the F_{420} content in *A. fulgidus* strain 7324 might be regulated by substrates.

Enzyme studies

Cell extracts of starch-grown cells were analyzed for enzyme activities involved in glucose degradation to pyruvate and pyruvate conversion to acetate. The enzymes found are listed in Table 1.

Enzymes of a modified Embden-Meyerhof pathway

ADP-dependent hexokinase

Cell extracts catalyzed the ADP-dependent phosphorylation of glucose to glucose 6-phosphate with a specific activity of 0.19 U/mg (50 °C). PP_i and ATP did not serve as phosphoryl donor, indicating the presence of an ADP-dependent hexokinase. The apparent K_m values for ADP and glucose were about 0.2 and 2.6 mM, respectively.

ADP-dependent 6-phosphofructokinase

Cell extracts catalyzed the ADP-dependent phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate

Table 1 Specific activities and apparent K_m values of enzymes involved in starch fermentation to acetate, and CO_2 in cell extracts of starch-grown *Archaeoglobus fulgidus* strain 7324. *BV* Benzylviologen, *CoA* coenzyme A, *Fd* ferredoxin, *F-1,6-BP* fructose 1,6-bisphosphate, *F-6-P* fructose 6-phosphate, *GAP* glyceraldehyde-3-phosphate, *Glc* glucose, *G-6-P* glucose 6-phosphate, *OR* oxidoreductase, *PEP* phosphoenolpyruvate, *2-PG* 2-phosphoglycerate, *3-PG* 3-phosphoglycerate, P_i inorganic phosphate, *n.d.* not determined

Enzyme activity	Temperature (°C)	Specific activity (U mg^{-1})	Apparent K_m (mM)
Hexokinase [Glc+ADP→G-6-P+AMP]	50	0.1	0.26 (Glucose) 0.2 (ADP)
Glucose-6-phosphate isomerase	80	0.06	4 (F-6-P)
6-Phosphofructokinase [F-6-P+ADP→F-1,6-BP+AMP]	50	0.11	1 (ADP) 3.3 (F-6-P)
Fructose-1,6-bisphosphate aldolase	50	0.01	4.2 (F-1,6-BP)
Triosephosphate isomerase	50	0.38	0.16 (GAP)
Glyceraldehyde-3-phosphate:Fd-OR [GAP+2 BV_{ox}→3-PG+2 BV_{red}]	50	0.07	0.28 (GAP)
Phosphoglycerate mutase	50	0.02	n.d.
Enolase	50	0.79	2 (2-PG)
Pyruvate kinase (PEP+ADP→Pyruvate+ATP)	50	0.13	3.3 (PEP) 0.6 (ADP)
Pyruvate:ferredoxin-oxidoreductase [Pyruvate+2 BV_{ox}+CoA→Acetyl-CoA+2BV_{red}+CO_2]	50	0.13	n.d.
Acetyl-CoA synthetase (ADP-forming) [Acetyl-CoA+ADP+P_i→Acetate+CoA+ATP]	50	0.3	0.015 (ADP) 0.3 (P_i) 0.023 (Ac-CoA)

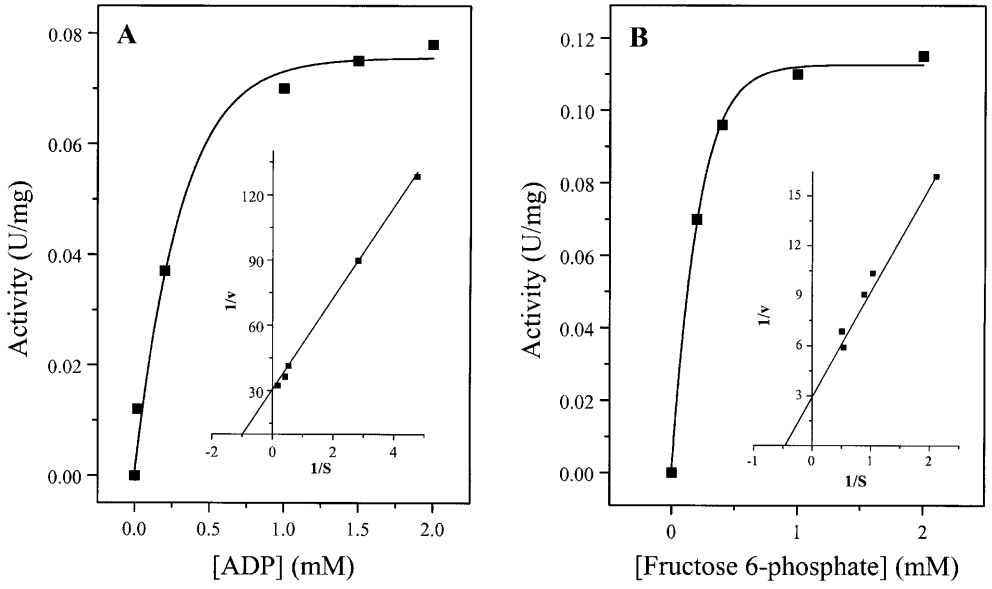

Fig. 2 A, B ADP-dependent 6-phosphofructokinase activity at 50 °C in cell extracts of starch-grown *A. fulgidus* strain 7324. Rate dependence on **A** the ADP concentration and **B** the fructose 6-phosphate concentration. *Inserts* Double reciprocal plots of the rates vs the corresponding substrate concentrations

with a specific activity of 0.1 U/mg (50 °C). PP_i and ATP did not serve as phosphoryl donors, indicating the presence of an ADP-dependent 6-phosphofructokinase. The apparent K_m values for ADP and fructose 6-phosphate were about 1 and 3.3 mM, respectively (Fig. 2A, B)

ATPase

Starch-grown cells contained high ATPase activity, catalyzing ADP formation from ATP at a specific activity of 1.5–2 U/mg at 50 °C. This enzyme caused an apparent "ATP stimulation" of both hexokinase and 6-phosphofructokinase activities – observed in crude extracts after an incubation period of 1–2 min at 50 °C – by hydrolyzing ATP to ADP, which is the actual phosphoryl donor of both kinases. Upon separation of hexokinase and phosphofructokinase activities from ATPase activity by gel filtration (see Materials and methods), "ATP stimulation" was eliminated.

Enzymes of glyceraldehyde 3-phosphate oxidation

Cell extracts of starch-grown *A. fulgidus* 7324 contained high activities of glyceraldehyde-3-phosphate: erredoxin oxidoreductase measured as GAP-dependent reduction of benzylviologen at a rate of about 0.1 U/mg (50 °C). The rate of benzylviologen reduction was not stimulated by the addition of 10 mM phosphate to phosphate-free extracts (<10 μM) and was not accelerated by the addition of arsenate (10 mM, K^+ salt), indicating that GAP oxida-

Table 2 Comparison of specific activities of enzymes (U/mg at 50 °C) and and content of F_{420} (nmol/mg protein) in lactate- and starch-grown cells of *A. fulgidus* strain 7324 at 50 °C. *Numbers in parentheses* indicate increase of activity (-fold) in starch-grown cells vs lactate-grown cells. – Not detectable

Enzyme	Lactate-grown cells	Starch-grown cells
ADP-dependent hexokinase	0.007	0.1 (14)
ADP-dependent 6-phosphofructokinase	0.01	0.11 (11)
Pyruvate kinase	0.028	0.130 (5)
Triosephosphate isomerase	0.38	0.38
Glyceraldehyde-3-phosphate:ferredoxin oxidoreductase	–	0.07
Acetyl-CoA synthetase (ADP-forming)	0.03	0.3 (10)
Glyceraldehyde-3-phosphate dehydrogenase	0.005	0.005
Adenylate kinase	2.6	2.6
Carbon monoxide dehydrogenase	0.12	0.11
F_{420} content	0.6	<0.03

tion does not proceed via 1,3-bisphosphoglycerate. Glyceraldehyde-3-phosphate dehydrogenase activity in starch-grown cells was 0.005 U/mg at 50 °C. Phosphoglycerate kinase activity could not be detected under the experimental conditions used. The data indicate that GAP:FdOR is the enzyme responsible for GAP oxidation to 3-phosphoglycerate during growth of *A. fulgidus* 7324 on starch.

Other enzyme activities of the Embden-Meyerhof pathway

Cell extracts of starch-grown *A. fulgidus* 7324 contained the following enzymes catalyzing conventional reactions: glucose-6-phosphate isomerase, fructose-1,6-bisphosphate aldolase, triosephosphate isomerase, phosphoglycerate mutase, enolase, and pyruvate kinase. The specific activities and some kinetic constants are given in Table 1.

Starch-specific induction of the modified Embden-Meyerhof pathway

Key enzymes of the modified Embden-Meyerhof pathway were also measured in lactate-grown cells. As shown in Table 2, the specific activities of ADP-dependent hexokinase, ADP-dependent 6-phosphofructokinase and pyruvate kinase, were 14-, 11- and 5-fold, respectively, higher in starch-grown cells than in lactate-grown cells. GAP:FdOR could not be detected in lactate-grown cells but showed high activities in starch-grown cells. These findings indicate that the catabolic enzymes of the modified Embden-Meyerhof pathway are induced under conditions of starch degradation.

From the enzyme data obtaine, we propose that glucose conversion to pyruvate during growth of *A. fulgidus* 7324 on starch proceeds via the modified Embden-Meyerhof pathway (see Fig. 3).

Enzymes of acetate formation from pyruvate

Cell extracts contained pyruvate ferredoxin oxidoreductase catalyzing the CoA-dependent reduction of benzylviologen as ferredoxin substitute at a rate of 0.13 U/mg (50 °C). Analyzing the enzymes of acetyl-CoA conversion to acetate in starch-grown cells, we could not detect activities of acetate kinase and phosphate acetyltransferase. Instead, extracts catalyzed the ADP- and P_i-dependent conversion of acetyl-CoA, to acetate, ATP, and CoA at a rate of 0.1–0.3 U/mg (50°), indicating the presence of an ADP-forming acetyl-CoA synthetase (ADP-ACS). Apparent K_m values for ADP (15 μM), P_i (300 μM) and acetyl-CoA (23 μM) were calculated from linear Lineweaver-Burk plots of the rate dependence on the substrate concentration. ADP-ACS activity in starch-grown cells, was up to ten-fold higher than in lactate-grown cells (0.03 U/mg at 50 °C) which do not form acetate, indicating induction of the enzyme under conditions of acetate formation. The data indicate that acetate formation from acetyl-CoA in the archaeon *A. fulgidus* 7324 is catalyzed by ADP-forming acetyl-CoA synthetase, which is the mechanism of acetate formation in all archaea tested so far (Selig et al. 1997).

Other enzyme activities in starch-grown cells of *A. fulgidus* 7324

Cell extracts contained NADPH:ferredoxin oxidoreductase, measured as NADPH-dependent reduction of benzylviologen as ferredoxin substitute, at a rate of 0.66 U/mg (60 °C). Starch-grown cells contained carbon monoxide dehydrogenase, determined as CO-dependent reduction of benzylviologen, at rates of about 0.1–0.2 U/mg (50 °C). Almost the same specific activities of carbon monoxide dehydrogenase were found in lactate-grown cells of *A. fulgidus* strain 7324 (Beeder et al. 1994).

Discussion

In the present communication, it was found that the archaeal hyperthermophilic sulfate reducer *Archaeoglobus fulgidus* strain 7324 can grow on starch and sulfate as carbon and energy source. Fermentation products and enzyme measurements indicated that starch was converted to acetate involving a modified Embden Meyerhof path-

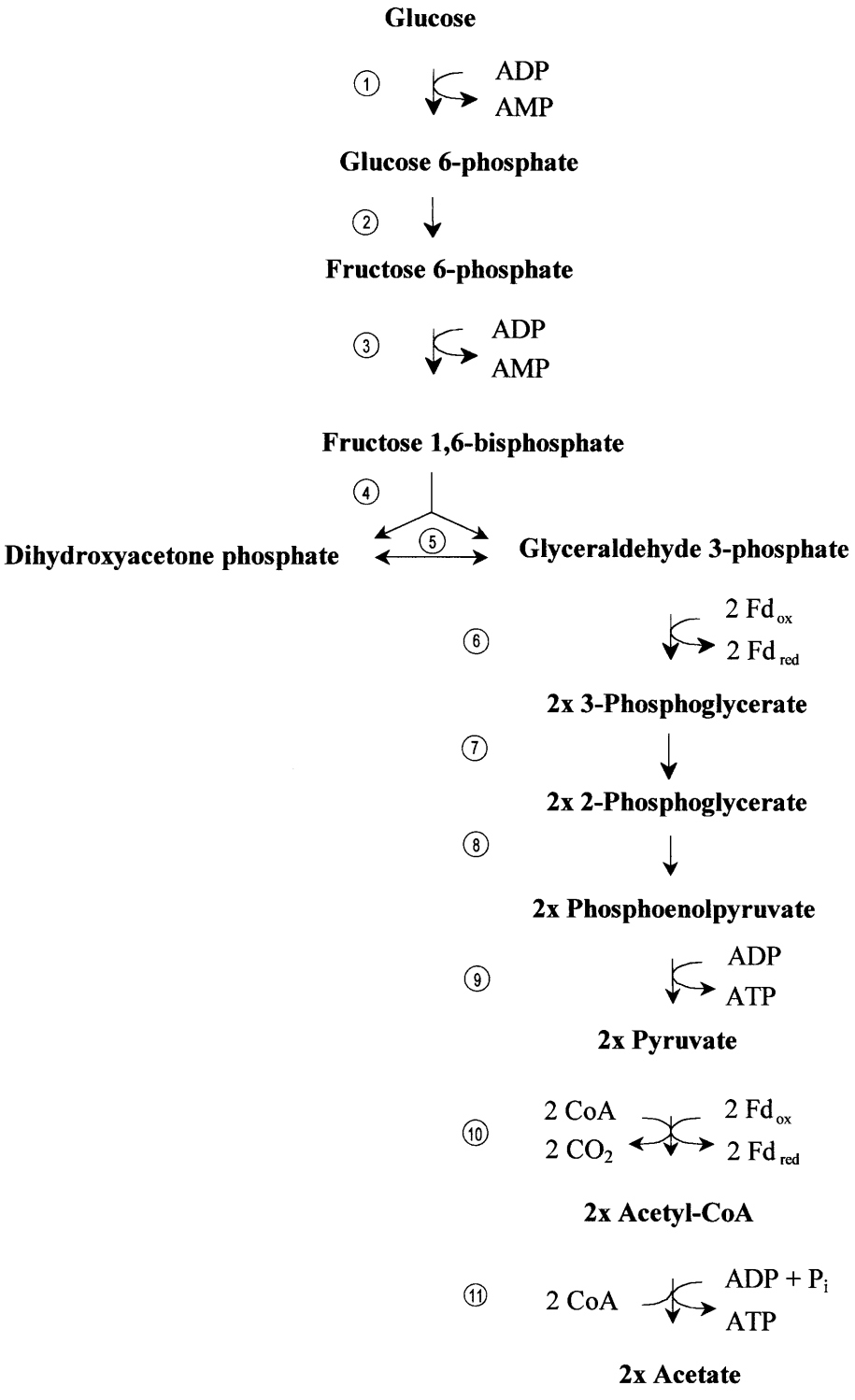

Fig. 3 Proposed pathway of starch degradation – via glucose – to acetate and CO_2 in *A. fulgidus* strain 7324. The enzymes of the modified Embden-Meyerhof pathway (enzymes *1–9*) and of pyruvate conversion to acetate and CO_2 (enzymes *10–11*) are shown. *1* ADP-dependent hexokinase, *2* glucose-6-phosphate isomerase, *3* ADP-dependent 6-phosphofructokinase, *4* fructose-1,6-bisphosphate aldolase, *5* triosephosphate isomerase, *6* glyceraldehyde-3-phosphate: ferredoxin oxidoreductase, *7* mutase, *8* enolase, *9* pyruvate kinase, *10* pyruvate:ferredoxin oxidoreductase, *11* ADP-forming acetyl-CoA synthetase. Fd_{ox} oxidized ferredoxin, Fd_{red} reduced ferredoxin

way and ADP-forming acetyl-CoA synthetase. This is the first report of utilization of starch in a sulfate-reducing prokaryote.

Sugar utilization by sulfate reducers

The ability to utilize sugars as electron donors for sulfate reduction appears to be a limited metabolic property of sulfate reducers; so far, only a few bacterial sulfate reducers have been reported to grow on sugars. *Desulfovibrio fructosovorans* grows on fructose and sulfate (Ollivier et al. 1988; Cord-Ruwisch et al. 1986); growth on fructose has also been reported for *Desulfotomaculum nigrificans* and *Desulfotomaculum geothermicum* (Klemps et al. 1985). For *Desulfovibrio simplex*, slow growth on glucose in the presence of peptone and yeast extract has been indicated (Zellner et al. 1989b). Utilization of several car-

bohydrates, including fructose and glucose, has been reported for *Desulfovibrio termitidis*, isolated from the hindgut of a termite (Trinkerl et al. 1990). So far, utilization of polymeric sugars, such as starch, by sulfate reducers has not been demonstrated.

As shown in this study, *A. fulgidus* strain 7324 can grow on starch and sulfate. Fermentation balances indicate that starch was incompletely oxidized with sulfate to acetate (2 mol acetate formed per mol glucose-equivalent oxidized). Incomplete oxidation of fructose at almost the same stoichiometry was also shown by *Desulfoviobrio fructosovorans* converting fructose (1 mol) with sulfate to 2 mol each of acetate and CO_2, and 1 mol of H_2S (Ollivier et al. 1989). Acetate formation was also found during growth of *D. simplex* on glucose (Zellner et al. 1989b) as well as during degradation of intracellular polyglucose reserves in *Desulfovibrio baculatus* (Stams et al. 1983) and *Desulfovibrio gigas* (Fareleira et al. 1997).

The incomplete oxidation of starch in *A. fulgidus* 7324 is of interest since lactate was found to be completely oxidized to 3 CO_2 by this organism. The formation of acetate from starch suggests that in starch-utilizing *A. fulgidus* cells the modified acetyl-CoA/carbon monoxide dehydrogenase pathway (Thauer and Kunow 1995) is not operative or is present only at low activity. The apparent absence of the deazaflavin F_{420} in starch-grown cells of *A. fugidus* 7324 might be correlated with this metabolic situation, since two F_{420}-dependent dehydrogenases, methylene tetrahydromethanopterin dehydrogenase and methylene tetrahydromethanopterin reductase, are involved in acetyl-CoA oxidation to 2 CO_2 via the modified acetyl-CoA/CO-DH pathway; these F_{420} dependent enzymes might be rate-limiting for acetyl-CoA oxidation. The first enzyme of the pathway, carbon monoxide dehydrogenase, showed almost the same specific activities in completely oxidizing lactate-grown cells, as compared to incompletely oxidizing starch-grown cells. This finding indicates that acetyl-CoA cleavage and the oxidation of enzyme-bound carbon monoxide (Thauer and Kunow 1995) are not the rate-limiting steps for acetyl-CoA oxidation in starch-grown *A. fulgidus* 7324.

The reasons for the metabolic switch from complete oxidation of lactate to incomplete oxidation of starch and the regulation of F_{420} content in *A. fulgidus* 7324 remains to be elucidated. Substrate-specific differences of both F_{420} content and activities of F_{420}-dependent methylene dehydrogenase and F_{420}-dependent methylene tetrahydromethanopterin reductase have been reported for *Archaeoglobus lithotrophicus* and *A. fulgidus* VC16. *A lithotrophicus* grown lithoautotrophically on H_2/CO_2 and sulfate contained significantly lower amounts of F_{420} content (<30%), and lower activities of F_{420}-dependent dehydrogenases (<5%) than measured in *A. fulgidus* VC16 grown organotrophically on lactate and sulfate. This was explained by the anabolic function of the carbon monoxide dehydrogenase/acetyl-CoA pathway in autotrophic CO_2 fixation in *A. lithotrophicus* as compared to the catabolic role of the pathway during complete lactate oxidation in *A. fulgidus* (Vorholt et al. 1995).

Enzymes of the modified Embden-Meyerhof pathway

The enzymes involved in starch degradation to glucose in *A. fulgidus* 7324 were not analyzed. In various other starch-degrading hyperthermophiles, both archaea and bacteria, a variety of amylolytic enzymes, such as α-amylases, and pullulanases, have been identified, purified, and characterized (for references, see Sunna et al. 1997; Lèvêque et al. 2000).

In starch-grown cells of *A. fulgidus* 7324, all enzymes of a modified Embden-Meyerhof (EM) pathway were detected converting glucose to pyruvate. This modified pathway contained the unusual enzymes ADP-dependent hexokinase and ADP-dependent phosphofructokinase and glyceraldehyde-ferredoxin oxidoreductase. Both ADP-dependent kinases have been found so far only in the hyperthermophilic archaea *Pyrococcus* and *Thermococcus* (Kengen et al. 1994; Selig et al. 1997; Ronimus et al. 1999). The enzymes from *P. furiosus* have been purified and the encoding genes have been identified in the complete sequenced genome of this organism (Kengen et al. 1995; Tuininga et al. 1999). Sequence comparison revealed homologous ORFs only in the genomes of other *Pyrococcus* strains and of the methanogen *Methanococcus jannaschii*, rather than in the genome of *Archaeoglobus fulgidus* VC16 (Tuininga et al. 1999). GAP:FdOR has been reported for *Pyrococcus* and *Thermococcus* species and for *Desulfurococcus amylolyticus* as part of the modified EM pathways in these organisms (Mukund and Adams 1995; Selig et al. 1997; van der Oost et al. 1998; de Vos et al. 1998). The proposed modified EM pathway involved in starch degration – via glucose – to pyruvate in shown in Fig. 3. The formal net ATP yield of this pathway, which involves GAP:FdOR catalyzing GAP oxidation to 3-phosphoglycerate without ATP formation, is zero: 2 mol ADP are converted to 2 mol AMP in the ADP-dependent hexokinase and 6-phosphofructokinase reactions. The regeneration of 2 mol ADP from 2 mol AMP requires the consumption of 2 mol ATP in the adenylate kinase reaction; 2 mol ATP are formed in the pyruvate kinase reaction. High activities of both adenylate kinase and inducible pyruvate kinase were found in starch-grown *A. fulgidus* 7324.

Starch-grown *A. fulgidus* 7324 contained pyruvate:ferredoxin oxidoreductase catalyzing pyruvate oxidation to acetyl-CoA. Acetate formation from acetyl-CoA was catalyzed by ADP-forming acetyl-CoA synthetase, in which 1 mol ATP/1 mol acetate is formed by the mechanism of substrate-level phosphorylation (see in Fig. 3).

The presence of both a modified EM pathway and ADP-forming acetyl-CoA synthetase in the archaeon *A. fulgidus* is in accordance with the current view that all archaea degrade sugars via modified glycolytic pathways and convert acetyl-CoA to acetate via ADP-forming acetyl-CoA synthetase (Selig et al. 1997). In contrast, in the domain of Bacteria, conventional EM (or Entner-Doudoroff) pathways are operative in sugar degradation, and acetate formation from acetyl-CoA is catalyzed by two enzymes, phosphate acetyltransferase and acetate ki-

nase. In accordance, all enzymes of the classical EM pathway and acetate kinase have been measured in the bacterial sulfate reducer *Desulfovibrio gigas* (Farelaira et al. 1997), which degrades the internal storage compound polyglucose to acetate. Acetate kinase and phosphate acetyl-transferase have also been measured in other sulfate reducers, forming acetate from acetyl-CoA as part of incomplete oxidation of organic compounds (see in Widdel and Hansen 1992). Thus, the domain-specific utilization of both different EM pathways and different enzymes of acetate formation is also valid for the sulfate-reducing prokaryotes.

Growth of the type strain *A. fulgidus* VC16 on starch or on any other sugar tested could not be demonstrated in this work. In extracts of lactate-grown cells of *A. fulgidus* VC16, no activities of hexokinase, 6-phosphofructokinase, and pyruvate kinase could be detected, and homologous genes encoding these enzymes were not found in the genome of *A. fulgidus* VC16. Therefore we conclude that the inability of *A. fulgidus* VC16 to grow on sugars might be due to the absence of the genes coding for the enzymes of the catabolic EM pathway. To test this hypothesis, we are currently purifying ADP-dependent hexokinase, ADP-dependent phosphofructokinase and pyruvate kinase from *A. fulgidus* strain 7324. Using the N-terminal amino acid sequences of the purified enzymes, it can then be decided whether the *A. fulgidus* VC16 genome contains genes (ORFs) coding for these kinases.

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References

Beeder J, Nilsen RK, Rosnes JT, Torsvik T, Lien T (1994) *Archaeoglobus fulgidus* isolated from hot north sea oil field waters. Appl Environ Microbiol 60:1227–1231

Beutler HO (1988) Starch. In: Bergmeyer H-U (ed) Methods of enzymatic analysis, vol VI. Verlag Chemie Weinheim, pp 2–10

Bode C, Goebell H, Stahler E (1968) Zur Eliminierung von Trubungsfehlern bei der Eiweissbestimmung mit der Biuretmethode. Z Klin Chem Klin Biochem 6:418–422

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

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

Cline JD, Spectrometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 14:454–458

Cord-Ruwisch R, Ollivier B, Garcia J (1986) Fructose degradation by *Desulfovibrio sp*. in pure culture and in coculture with *Methanospirillum hungatei*. Curr Microbiol 13:285–289

Cordwell SJ (1999) Microbial genomes and "missing" enzymes: redefining biochemical pathways. Arch Microbiol 172:269–279

Danson MJ (1993) Central metabolism of the archaea. New Compr Biochem 26:1–24

De Vos WM, Kengen SW, Voorhorst WG, van der Oost J (1998) Sugar utilization and its control in hyperthermophiles. Extremophiles 2:201–205

Fareleira P, Legall J, Xavier AV, Santos H (1997) Pathways for utilization of carbon reserves in *Desulfovibrio gigas* under fermentative and respiratory conditions. J Bacteriol 179:3972–3980

Hansen T, Schönheit P (2000) Purification and properties of the first archaeal ATP-dependent 6-phosphofructokinase, an extreme thermophilic non-allosteric enzyme, from the hyperthermophilic *Desulfurococcus amylolyticus*. Arch Microbiol 173: 103–109

Kengen SW, de Bok FA, van Loo ND, Dijkema C, Stams AJ, De Vos WM (1994) Evidence for the operation of a novel Embden-Meyerhof pathway that involves ADP-dependent kinases during sugar fermentation by *Pyrococcus furiosus*. J Biol Chem 269:17537–17541

Kengen SW, Tuininga JE, de Bok FA, Stams AJ, De Vos WM (1995) Purification and characterization of a novel ADP-dependent glucokinase from the hyperthermophilic archaeon *Pyrococcus furiosus*. J Biol Chem 270:30453–30457

Klemps R, Cypionka H, Widdel F, Pfennig N (1985) Growth with hydrogen, and further physiological characteristics of *Desulfotomaculum* species. Arch Microbiol 143:203–208

Klenk HP, Clayton RA, Tomb JF, White O, Nelson KE, Ketchum KA, Dodson RJ, Gwinn M, Hickey EK, Peterson JD, Richardson DL, Kerlavage AR, Graham DE, Kyrpides NC, Fleischmann RD, Quackenbush J, Lee NH, Sutton GG, Gill S, Kirkness EF, Dougherty BA, McKenney K, Adams MD, Loftus B, Venter JC (1997) The complete genome sequence of the hyperthermophilic, sulphate- reducing archaeon *Archaeoglobus fulgidus.* Nature 390:364–370

Lévêque E, Janecek S, Haye, Belarbi A (2000) Thermophilic archaeal amylolytic enzymes. Enzyme Microb Technol 26:3–14

Möller-Zinkhan D, Börner G, and Thauer RK (1989) Function of methanofuran, tetrahydromethanopterin, and coenzyme F_{420} in *Archaeoglobus fulgidus*. Arch Microbiol 152, 362–368

Mukund S, Adams MW (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 270:8389–8392

Noll F (1988) L-(+)-Lactate. In: Bergmeyer H-U (ed) Methods of enzymatic analysis, vol VII. Verlag Chemie Weinheim, pp 582–588

Ollivier B, Cord-Ruwisch R, Hatchikian EC, Garcia JL (1988) Characterization of *Desulfovibrio fructosovorans* sp. nov. Arch Microbiol 149:447–450

Ronimus RS, Koning J, Morgan HW (1999) Purification and characterization of an ADP-dependent phosphofructokinase from *Thermococcus zilligii*. Extremophiles 3:121–129

Schäfer T, Schönheit P (1991) Pyruvate metabolism of the hyperthermophilic archaebacterium *Pyrococcus furiosus*. Acetate formation from acetyl-CoA and ATP synthesis are catalysed by an acetyl-CoA synthetase (ADP-forming). Arch Microbiol 155:366–377

Schäfer T, Schönheit P (1992) Maltose fermentation to acetate, CO_2 and H_2 in the anaerobic hyperthermophilic archaeon *Pyrococcus furiosus*: evidence for the operation of a novel sugar fermentation pathway. Arch Microbiol 158:188–202

Schäfer T, Selig M, Schönheit P (1993) Acetyl-CoA synthethase (ADP-forming) in archaea, a novel enzyme involved in acetate and ATP synthesis. Arch Microbiol 159:72–83

Schönheit P, Schäfer T (1995) Metabolism of hyperthermophiles. World J Microbiol Biotechnol 11:26–57

Schönheit P, Keweloh H, Thauer RK (1981) Factor F_{420} degradation in growing *Methanobacterium thermoautotrophicum* during exposure to oxygen. FEMS Microb Lett 12:347–349

Schröder C, Selig M, Schönheit P (1994) Glucose fermentation to acetate, CO_2 and H_2 in the hyperthermophilic eubacterium *Thermotoga maritima*: involvement of the Embden-Meyerhof pathway. Arch Microbiol 161:460–470

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

Stams AJ, Veenhuis M, Weenk GH, Hansen TA (1983) Occurence of polyglucose as a storage polymer in *Desulfovibrio species* and *Desulfobulbus propionicus*. Arch Microbiol 136:54–59

Stetter KO (1988) *Archaeoglobus fulgidus* gen. nov., sp. nov.: a new taxon of extremely thermophilic archaebacteria. Syst Appl Microbiol 10:172–173

Stetter KO (1996) Hyperthermophilic procaryotes. FEMS Microbiol Rev 18:149–158

Stetter KO (1999) Extremophiles and their adaptation to hot environments. FEBS Lett 452:22–25

Stetter KO, Lauerer G, Thomm M, Neuner A (1987) Isolation of extremely thermophilic sulfate reducers: evidence for a novel branch of archaebacteria. Science 236:822–824

Sunna A, Moracci M, Rossi M, Antranikian G (1997) Glycosyl hydrolases from hyperthermophiles. Extremophiles 1:2–13

Thauer RK, Kunow J (1995) Sulfate-reducing Archaea. In: Clark N (ed) Biotechnology handbook. Plenum, London, pp 33–48

Trinkerl M, Breunig A, Schauder R, König H (1990) *Desulfovibrio termitidis* sp. nov., a carbohydrate-degrading sulfate-reducing bacterium form the hindgut of a termite. Syst Appl Microbiol 13:372–377

Tuininga JE, Verhees CH, van der Oost J, Kengen SW, Stams AJ, De Vos WM (1999) Molecular and biochemical characterization of the ADP-dependent phosphofructokinase from the hyperthermophilic archaeon *Pyrococcus furiosus*. J Biol Chem 274:21023–21028

Van der Oost J, Schut G, Kengen SW, 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

Vorholt J, Kunow J, Stetter KO, Thauer RK (1995) Enzymes and coenzymes of the carbon monoxide dehydrogenase pathway for autotrophic CO_2 fixation in *Archaeoglobus lithotrophicus* and the lack of carbon monoxide dehydrogenase in the heterotrophic *A. profundus*. Arch Microbiol 163:112–118

Widdel F, Hansen TA (1992) The dissimilatory sulfate- and sulfate-reducing bacteria. In: Balows A, Trüper HG, Dworkin M, et al (eds) The prokaryotes, vol 1. Springer, Berlin Heidelberg New York, pp 583–624

Zellner G, Stackebrandt E, Kneifel H, Messner P, Sleytr UB, Conway de Macario E, Zabel H-P, Stetter KO, Winter J (1989a) Isolation and characterization of a thermophilic, sulfate reducing archaebacterium, *Archaeoglobus fulgidus* strain Z. Syst Appl Microbiol 11:151–160

Zellner G, Messner P, Kneifel H, Winter J (1989b) *Desulfovibrio simplex* spec. nov., a new sulfate-reducing bacteria from a sour whey digester. Arch Microbiol 152:329–334