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ORIGINAL PAPER

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Regulation of expression of the arabinose and glucose transporter genes in the thermophilic archaeon *Sulfolobus solfataricus*

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Abstract Sugar uptake in *Sulfolobus solfataricus*, a thermoacidophilic archaeon, occurs through high-affinity binding of protein-dependent ABC transporters. We have investigated the expression patterns of two sugar transport operons, that is, the glucose and arabinose transporters. Analysis of the *araS* promoter activity, and the mRNA and protein levels in *S. solfataricus* cells grown on different carbon sources showed that expression of the arabinose transporter gene cluster is highly regulated and dependent on the presence of arabinose in the medium. Glucose in the growth medium repressed the expression of the arabinose transport genes. By means of primer extension, the transcriptional start site for the arabinose operon was mapped. Interestingly, expression of the arabinose transporter is down-regulated by addition of a selective set of amino acids to the medium. Expression of the glucose transporter genes appeared constitutive. These data confirm the earlier observation of a catabolite repression-like system in *S. solfataricus*.

Keywords Archaea · *Sulfolobus* · Transport · Catabolite repression · Regulation

Introduction

For effective utilization of substrates from the environment, microbial cells employ catabolite repression. Commonly, the presence of glucose prevents the induction of the expression of enzymes linked to the utilization of substrates other than glucose, as a carbon and energy source. Recently, the phenomenon of catabolite repression was also reported in the hyperthermophilic archaeon *Sulfolobus solfataricus* (Haseltine et al. 1996, 1999a). Several of the characteristic features were observed such as the transient repression by glucose, carbon source hierarchy and a global mode of regulation. Coordinated regulation of expression of the genes involved in carbon and energy metabolism (carbohydrate utilization) has been shown. The activities of α-glucosidase, β-glycosidase, α-amylase, encoded by the *malA*, *lacS* and *amyA* genes, respectively, responded to the presence of supplementary carbon sources such as amino acids (Haseltine et al. 1999a; Hoang et al. 2004). These amino acids were subdivided into two groups according to the effect they evoked. Amino acids with a repressive effect are alanine, asparagine, aspartate, and arginine. The amino acids glutamine, glutamate, glycine, histidine, and leucine showed no effect (Haseltine et al. 1999a). Recently, the presence of a sequence modulating expression of the genes involved in the catabolite repression in *S. solfataricus* has been demonstrated (Haseltine et al. 1999b). The gene called *car* (catabolite repression) seems to be engaged in the regulation of the expression of β-glycosidase and α-amylase, but not of α-glucosidase (Haseltine et al. 1999b). *Car* encodes or modulates a factor that affects *lacS* expression, and it is possible that *car* produces a positively acting regulatory factor (Hoang et al. 2004).

Sugar metabolism has been intensively studied in *S. solfataricus* (Albers et al. 2004; Haseltine et al. 1996, 1999a), but remarkably little is known about how sugar metabolism and transport are regulated and coordinated in archaea. Archaeal gene regulation resembles either bacterial or eukaryal strategies, while some systems show unique features. The mechanisms of replication, transcription (which resembles polymerase II type), and translation, are eukaryal-like (Thomm 1996; Grabowski and Kelman 2003; Bell and Jackson 2000, 2001). In

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contrast, the regulation of gene expression is mostly similar to bacterial schemes, and this group of archaeal regulators represent, for instance, Sa-Lrp from *S. acidocaldarius* (Enoru-Eta et al. 2000), LrpA from *P. furiosus* (Brinkman et al. 2000) or LysM from *S. solfataricus* (Brinkman et al. 2002), an archaeal homologue of the Lrp/AsnC family; TrmB, transcriptional regulators of the trehalose/maltose ABC transporters from *P. furiosus* and *T. litoralis* (Lee et al. 2003, 2005) and others. There are also a few eukaryal types of regulators found in archaea represented by the Sir2 and GvpE proteins. Sir2 is a homologue of eukaryal Sir2, that is known to acetylate ALBA (acetylation lowers binding affinity, formerly called Sso10b), one of the chromatin associated proteins, antagonizing the repressive capacity of this protein (Bell et al. 2002). GvpE is a transcriptional activator of *gvp* genes, which resembles a basic leucine zipper (bZIP) protein (Gregor and Pfeifer 2001).

In *S. solfataricus* transport of glucose and arabinose is mediated by high-affinity binding of protein-dependent ABC transporters (Albers et al. 1999). Both transporters consist of four genes (*glcSTUV* and *araSTUV*, respectively) that are organized in an operon: a substrate binding protein, two permeases and an ATPase, which forms a functional homodimer (Albers et al. 1999; Elferink et al. 2001). We have analysed the effect of different carbon sources on the regulation of the expression of the glucose and arabinose transport genes of *S. solfataricus*. Expression levels of the two operons were studied on the mRNA and protein level and was confirmed by *araS* promoter fusion studies. The data show that the *ara* operon is highly expressed when arabinose is present in the medium, and further demonstrate that this system is down-regulated by the presence of alternative carbon sources in the medium.

Experimental procedures

Strains and culture conditions

S. solfataricus P2 (DSM1617) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkultur (Braunschweig, Germany). *S. solfataricus* PH1-16 *pyrEF* mutant was isolated by Martusewitsch et al. (2000). Cells were grown aerobically at 80°C in a defined medium (Brock et al. 1972) that was adjusted to pH 3 with sulphuric acid and supplemented with either 0.4% (w/v) of sugars (arabinose or glucose), 0.4% tryptone, and/or amino acids (5 or 20 mM) as a sole carbon and energy source. The rich medium contained 0.4% tryptone, 0.2% sucrose, and 0.2% yeast extract. Growth was monitored spectrophotometrically at 600 nm. For optimal growth on minimal medium containing only sugars as a carbon source *S. solfataricus* P2 cells were first grown on 0.2% tryptone supplemented with 0.4% arabinose or 0.4% glucose. Cells were next transferred to fresh arabinose or glucose minimal media, and this was repeated twice to ensure that no proteins related to other metabolic routes were still present.

Preparation of *S. solfataricus* membranes

Cells were resuspended in 20 mM MES-HCl, 100 mM NaCl (pH 6.5) and sonicated for seven cycles for 15 s on and 45 s off (Soniprep 150, LA Abcoude). Unbroken cells were removed by low-spin centrifugation at 16,100 x *g* for 2 min. Membranes were collected by high-spin centrifugation at 95,000 x *g* for 45 min at 4°C. The membrane pellet was resuspended in 20 mM MES-HCl, 100 mM NaCl (pH 6.5), frozen in liquid nitrogen and stored at −80°C.

Western blot analysis

Western blot analysis was performed using PVDF membranes. Total protein was determined using DC Protein Assay (BioRad). Typically, 20 µg of membrane protein was loaded on the SDS-PAGE per lane. Proteins were detected with antibodies directed against AraV and GlcV that were raised against purified recombinant proteins in chickens or rabbits, respectively (Agrisera, Sweden). Secondary antibodies were directed against chicken and rabbit antibodies, and conjugated with alkaline phosphatase (Sigma). Blots were developed by chemiluminescence using CDP-Star (Roche Applied Science) and visualized on a Lumi Imager (Roche Applied Science).

Total RNA isolation and Northern analysis

Total RNA was isolated from exponentially growing *S. solfataricus* P2 cells using the TRIZOL Reagent (Gibco BRL Life Technologies, Breda, The Netherlands). For Northern blot analysis, 20 µg of total RNA was separated on 1.1% (v/v) formaldehyde agarose gels, and transferred to Zeta-probe membrane (BIORAD, Veenendaal, The Netherlands) by capillary blotting. Ribosomal RNA stained with methylene blue was used as an internal control for sample loading. Probes for *araS* (forward: 5′- tctggcgctgaaggtggata -3′; reverse 5′- tataacgtaaataccttgtg -3′) and *glcS* (forward 5′- ctgatagttgataaacgaag -3′; reverse 5′- ggcaatctatgtcatgggaa -3′) were DIG-labelled using PCR on genomic DNA of *S. solfataricus* (PCR DIG labelling mix^{PLUS}, Roche). Detection was performed with DIG-AP antibodies (Boehringer Mannheim, Germany) and CDP-Star (Tropix Inc., Bedford, USA). Primers were designed according to the genome sequence of *S. solfataricus* P2 (http://www-archbac.u-psud.fr/projects/sulfolobus).

Construction of virus vector for promoter studies

pSVA5 (Albers et al. 2006), a pUC18 derivative, contained the *pyrEF* genes for complementation and

the *lacS* gene (β-galactosidase) under the control of the *araS* promoter region of *S. solfataricus*. The promoter region included 241 bp upstream of the *araS* start codon and the start codon of *lacS* coincided with the former start codon of *lacS*. To transfer the *araS* promoter – *lacS* cassette to the virus vector, pSVA5 was digested with BlnI and EagI and the insert was ligated into pMJ02 (Jonuscheit et al. 2003) cut with the same enzymes, resulting in pSVA9. Single transformants of *S. solfataricus* PH1-16 with pSVA9 were obtained as described before (Jonuscheit et al. 2003).

Primer extension

Transcriptional start site of *araS* in *S. solfataricus* P2 was mapped using the primer extension procedure. Fifteen picomoles of a gene-specific primer (5′-cagcaattgctgcaattatg-3′) was incubated with 10 µg of total RNA and annealing buffer at 70°C for 10 min and annealing was carried out by cooling the reaction from 65°C down to 45°C in 2 min increments. Extensions were made in a total volume of 20 µl using reverse transcriptase (Fermentas, Hanover, USA) by adding the manufacturer's reaction buffer, 20 µM dNTPs, 20 U RNase inhibitor and incubation at 42°C for 1 h. The enzyme was inactivated at 70°C for 15 min followed by phenol-chloroform extraction and ethanol precipitation. The products were analysed by PAGE using sequencing reactions as size marker.

β-galactosidase activity assay

β-galactosidase activity of crude extracts from transformants was done as described before (Jonuscheit et al. 2003), except that cells were lysed by ultrasonication.

Results

Expression of the glucose and arabinose transporter genes in S. solfataricus P2 grown on different sugars

The arabinose and glucose transport genes are organized in operon-like structures (Fig. 1a). Both operons contain the structural genes that encode a membrane-bound substrate-binding protein, two permease domains and a cytoplasmic ATPase (Elferink et al. 2001). In contrast to the arabinose operon, in which all genes are transcribed in the same direction, the binding protein of the glucose transporter (*glcS*) is transcribed in an opposite direction compared to the permease and ATPase gene (Albers et al. 1999).

S. solfataricus was grown on different carbon sources for several generations to monitor the protein expression levels of the ATPases, AraV and GlcV, of the arabinose and glucose ABC transporters, respectively. Membranes were isolated from cells grown to OD_{600} 0.4 and protein levels were detected using antibodies directed against AraV and GlcV (Fig. 1b). Membranes isolated from the cells grown on arabinose showed high levels of AraV, whereas the protein was essentially absent in membranes

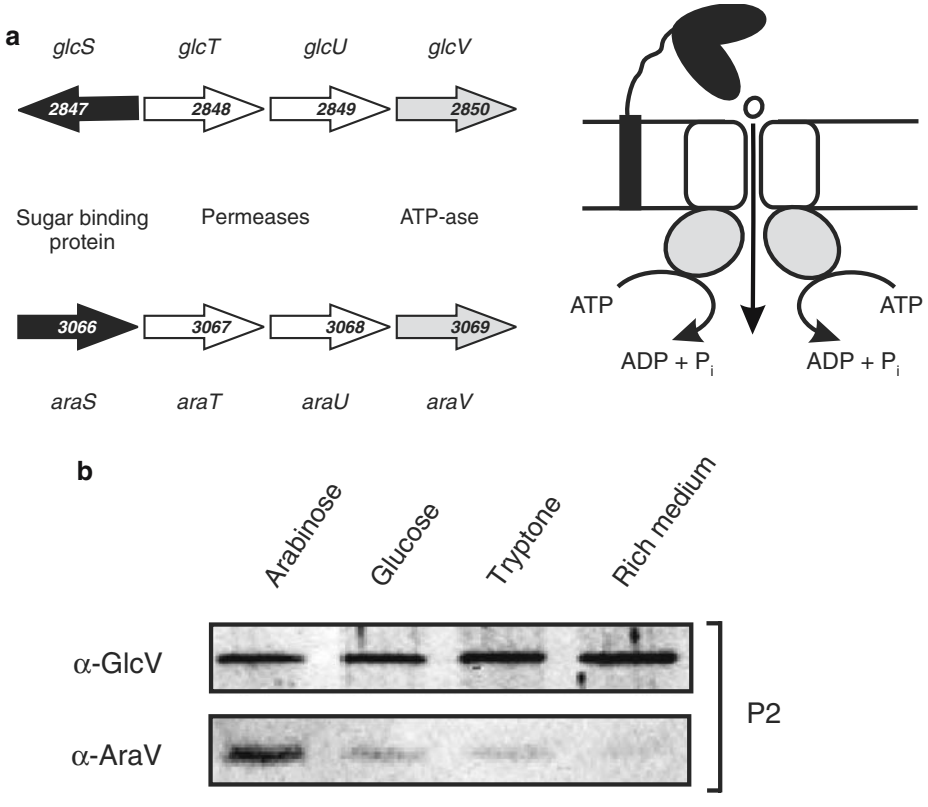

Fig. 1 Transport operon composition and analysis of expression levels of AraV and GlcV in *S. solfataricus* P2. **a** Operon composition of genes specifying subunits of the glucose and arabinose transporter on the *right* shows a schematic overview of the ABC sugar transporter organization. **b** *S. solfataricus* P2 was grown on minimal medium containing either arabinose, glucose, tryptone or rich medium. Membrane proteins were separated on SDS-PAGE and the AraV and GlcV expression levels were visualized by immunodetection

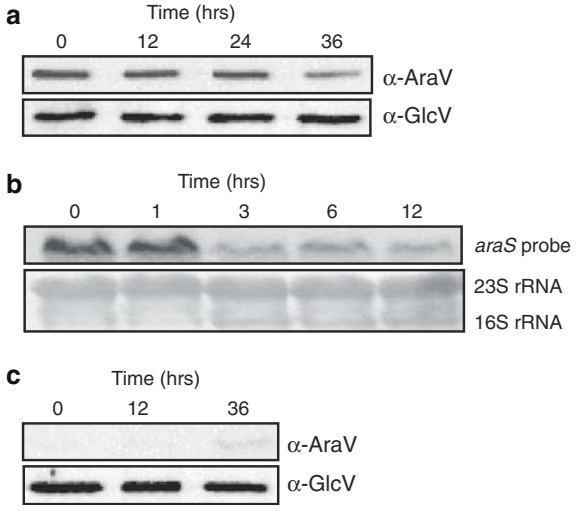

Fig. 2 **a** AraV and GlcV expression patterns in the *S. solfataricus* P2 cells pre-grown on arabinose after addition of glucose at the time point 0 with cells at an OD_{600} of 0.4. **b** Northern blot analysis of *araS* transcript expression levels using a specific 400 bp DIG-labelled probe and using cells that were pre-grown on arabinose. At time point 0, glucose was added. As a control for sample loading 23 and 16S rRNA levels were visualized on the same membrane by methylene blue staining before immunodetection (*lower panel*). **c** AraV and GlcV protein levels in the *S. solfataricus* P2 cells pre-grown on glucose and after addition of arabinose at the time point 0 using cells at an OD_{600} of 0.4

isolated from cells grown on glucose, tryptone or on yeast extract based rich medium. In contrast, the expression of GlcV remained at the same levels under all tested growth conditions.

When 0.2% glucose was added to a *S. solfataricus* culture pre-grown on 0.2% arabinose, the levels of GlcV remained stable whereas the levels of AraV slowly diminish after 36 h of continued growth (Fig. 2a). Northern blotting showed that *araS* mRNA levels dropped already after 1 hr upon the addition of glucose (Fig. 2b) (Haseltine et al. 1999a). Therefore, it seems that the AraV protein remains fairly stable within this 36-h period, while the loss of protein is most likely due to the dilution effect caused by the continued growth. On the other hand, the addition of 0.2% arabinose to *S. solfataricus* cells pre-grown on glucose had no effect on either the AraV or GlcV levels, suggesting a preference of *S. solfataricus* to utilize glucose rather than arabinose when present in the medium simultaneously (Fig. 2c).

Transcript analyses

The arabinose and glucose transport genes are organized in operon structures. Both operons consist of four ORFs: *araS*/*glcS* that encode the arabinose or glucose binding protein, respectively, *araTU*/*glcTU*, the permease domains located in the cytoplasmic membrane and the ATPases *araV*/*glcV*. Expression of the *araS* and *glcS* genes was tested by Northern blotting. Total RNA was isolated from cells grown under the conditions described above. High levels of *araS* were observed only when cells were grown in the presence of arabinose, whereas *glcS* was expressed under all tested growth conditions (Fig. 3b).

Analysis of the promoter region of the glucose transporter suggests the presence of two promoters that drive transcription into two directions, that is, transcription start sites are found for *glcS* and for the transporter genes *glcT*, *glcU* and *glcV* (Fig. 3a). Northern analysis indicates the presence of a single transcript for the *glcS* gene of 1,662 bp long (Fig. 3b), while for *glcT, U* and V a transcript of 2,778 bp seems to exist based on the Northern blotting results using a *glcV*- or *glcT*-derived probe (data not shown). However, we were not able to detect any signal when using a *glcU* probe, most probably due to a very low CG content of this gene. The sequence analysis of the *ara* operon also revealed the presence of two promoter regions (Fig. 3a), although the activity of only one, located upstream of *araS*, has been confirmed experimentally (see next). The size of the *araS* transcript indicates that the binding protein is transcribed as a single ORF as the corresponding transcript has a length of 1,860 bp that can only accommodate this gene (Fig. 3b). Only *araV*, and not *araT* and *araU*, could be detected on Northern blots and a transcript was visible at around 2,800 bp (data not shown). The latter suggests the presence of a *araTUV* transcript as it corresponds with the predicted size of 2,853 bp. In both cases, we could not detect a "full length" transcript spanning all four ORFs of *glcSTUV* (predicted size is 4,440 bp) and *araSTUV* (predicted size is 4,713 bp). The precise transcription start of *araS* was determined by primer extension analysis (Fig. 5). It was mapped to a G that was six nucleotides upstream of the translational start codon (ATG) of the *araS* gene and 29 nucleotides downstream of a canonical TATA-box (Reiter et al. 1990). Attempts to detect a transcription start side for the *araT* gene failed with this method.

Regulation of araS in an *in vivo* reporter gene system

In order to study the regulation of *araS* in more detail we employed the reporter gene system of *S. solfataricus* recently developed in our laboratory (Jonuscheit et al. 2003). *LacS*, a gene encoding *β*-galactosidase of *S. solfataricus*, was cloned behind a 241 bp region upstream of the start codon of *araS* and inserted into the virus-based shuttle-vector pMJ03 (Jonuscheit et al. 2003). The resulting plasmid pSVA9 was electroporated into the *pyrEF* auxotroph *S. solfataricus* strain PH1-16 (Martusewitsch et al. 2000). Single transformants were isolated and analysed by Southern analysis, to verify the presence of the vector (data not shown). *LacS* expression in single transformants was determined after growing cells on tryptone medium and subsequent transfer to media containing either tryptone or tryptone supplemented with arabinose, glucose, or maltose

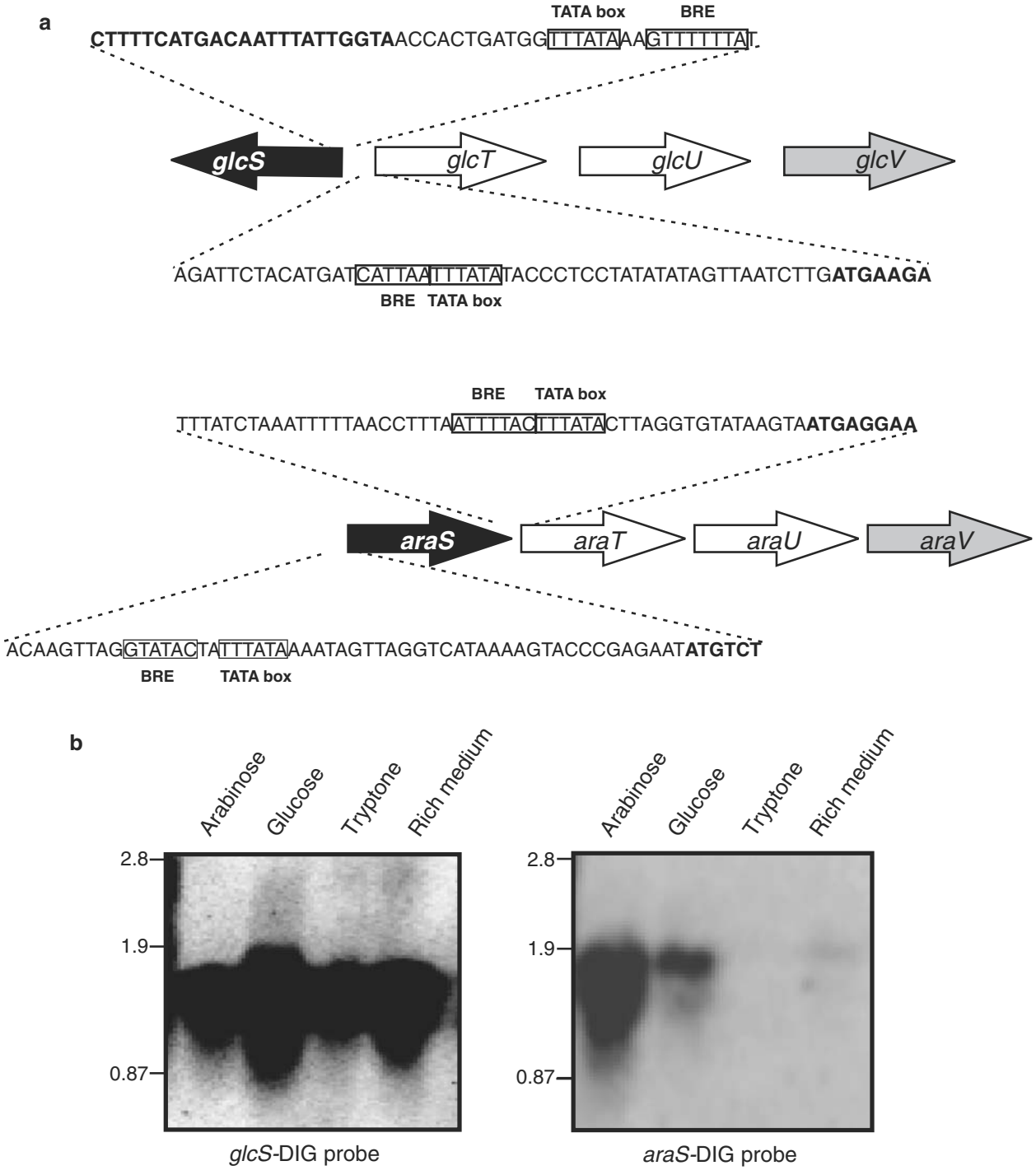

Fig. 3 Putative promoter regions and Northern blot analysis of mRNA levels of *glcS* and *araS* in *S. solfataricus* P2 cells. **a** Depiction of the putative promoter regions of the glucose and arabinose transport system. **b** *S. solfataricus* P2 was grown on minimal medium containing either arabinose, glucose, tryptone or rich medium. *glcS* and *araS* mRNA levels were detected by DIG immunolabelling

(Fig. 4). Addition of arabinose to pSVA9 single transformants grown on tryptone resulted in an increase in the LacS activity from 200 U/mg protein up to a maximum of 3,500 U/mg protein. In the absence of arabinose, the activity remained low, that is, at around 500 U or below under all other growth conditions tested (Fig. 4). The expression pattern therefore confirms the results obtained on the AraV expression levels in *S. solfataricus* membranes showing high induction when arabinose is added to the medium.

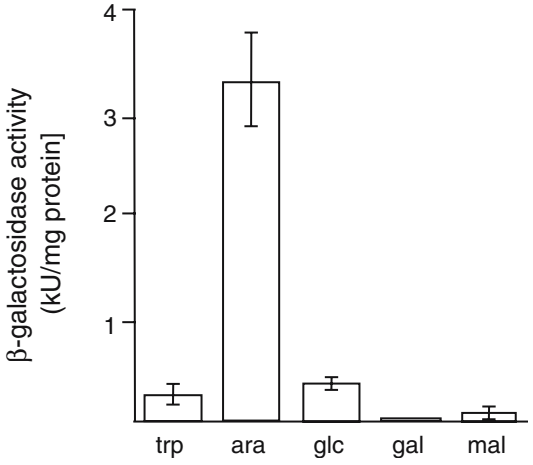

Fig. 4 Specific *β*-galactosidase activities in single pSVA9 transformants (*araS*-promoter + *β*-galactosidase as reporter) grown on tryptone medium (Tryp) or on arabinose (Ara), Glucose (Glc), Galactose (Gal) or Maltose (Mal) supplemented tryptone medium. Means and standard deviations were obtained from ten independent cultures/measurements. Each sugar was supplemented to an absolute amount of 0.4% (w/v) and *β*-galactosidase activity kinetics from crude extracts of cells from late log phase (OD_{600} 0.2–0.6) was determined. Glucose-containing cultures were incubated for several days as the growth rate with glucose as a sole carbon source is considerably lower as for the other sugars

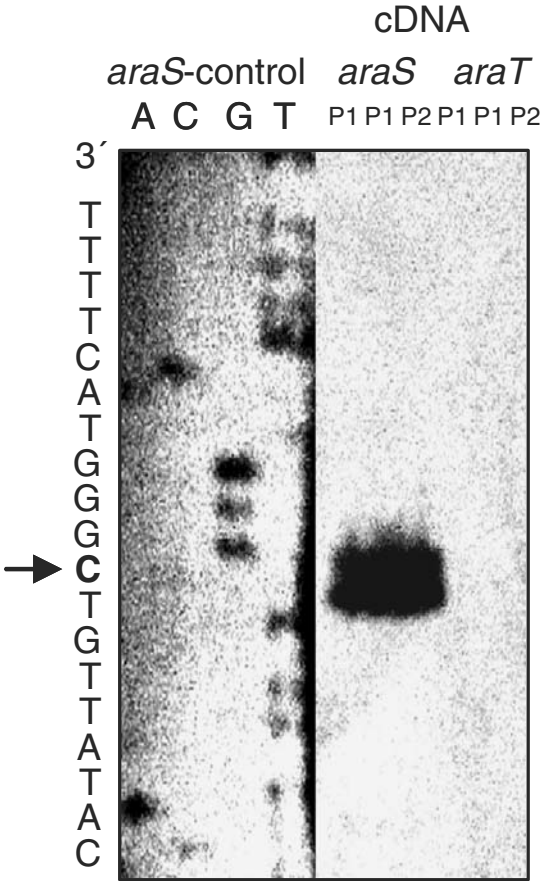

Fig. 5 Mapping of the transcription start of *ara*S by primer extension. Total RNA was isolated from *S. solfataricus* P1/P2 grown in Brocks's minimal medium containing 0.4% arabinose as a sole carbon source. cDNA was used for primer extension experiments to determine transcription initiation start sites of *araS* and *araT*. Two samples were applied for *S. solfataricus* P1 and for P2 in each case. A corresponding sequence reaction of the *araS* gene was run in parallel (*left side*)

Amino acids affect the expression levels of AraV and GlcV

The expression of various genes related to sugar metabolism in *S. solfataricus* has been shown to respond to the presence of amino acids in the growth medium (Haseltine et al. 1999a). In these studies, aspartate was found to be one of the most effective repressing amino acids whereas glutamate showed no inhibitory effect. To test the influence of amino acids on the expression levels of the arabinose transporter, *S. solfataricus* cells were grown on minimal medium with arabinose as the sole carbon source. Exponentially growing cells were transferred to media containing arabinose and either 5 or 20 mM aspartate. The AraV level was determined by western blot analysis at various time points after addition of the amino acid to the growth medium. Aspartate showed a strong repressing effect that was already evident after 6 h of growth (Fig. 6a). At low aspartate concentration (5 mM), the AraV level increased again after 36 h. This suggests that upon depletion of aspartate, the expression of the arabinose operon is reactivated (Fig. 6a). Similarly, glutamate also showed a marked repressing effect.

In order to determine which amino acids cause this repression, all amino acids were tested at a concentration of 20 mM. Samples were taken after 3 h of growth in the presence of arabinose and the indicated amino acids, and both total mRNA and membranes were isolated. mRNA analysis with the *araS* probe after 3 h shows that five of the amino acids, arginine, lysine, phenylalanine, proline, and tryptophan, had no or little effect on the expression levels, whereas alanine, aspartate, asparagine, cysteine, glutamate, leucine, threonine, tyrosine, and valine showed the most pronounced effect (Fig. 7a). After 3 h of growth, AraV protein levels remained mostly unaltered (Fig. 7b). None of the amino acids caused a significant change in the expression level of GlcV (data not shown), which further indicates that the glucose transporter is constitutively expressed.

Discussion

Here, we report on the growth condition-dependent expression of the glucose and arabinose transporters in *S. solfataricus*. Uptake of both arabinose and glucose involves a high-affinity membrane-bound binding protein and membrane-embedded ABC transporters with a permease and ATPase domain (Elferink et al. 2001; Albers et al. 1999). We demonstrated that the expression of the arabinose-binding protein, AraS, and the ATPase,

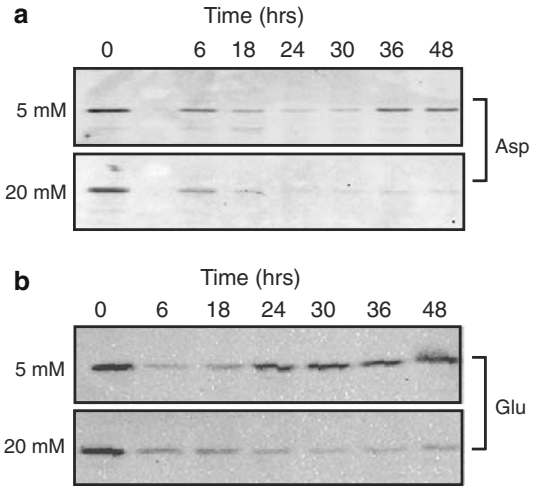

Fig. 6 Effect on amino acids on the AraV protein levels in membranes of arabinose-grown *S. solfataricus* P2. Cells were grown on arabinose and at time point 0 and an OD_{600} of 0.4 either aspartate **(a)** or glutamate **(b)** was added at 5 or 20 mM concentration as indicated. Growth was continued for the indicated times and the AraV levels in the membranes were detected by immunoblotting

AraV, is strongly dependent on the presence of arabinose in the growth medium. Studies with a reporter gene system consisting of an *araS* promoter-*lacS* fusion construct confirm this expression pattern and show that induction can results in at least sixfold increase in expression. Strikingly, expression of the arabinose operon genes is highly responsive to a certain group of amino acids. Alanine, arginine, aspartate, asparagine, glutamate and cysteine repress the *araS* expression. In contrast to the arabinose transporter, expression of the glucose transporter appeared high under all tested conditions. Neither the presence nor absence of glucose or the addition of amino acids to the growth medium had any effect on the expression. It thus appears that the arabinose transporter expression is regulated whereas the glucose transporter appears constitutive. One possible explanation for this distinct difference in regulation may relate to the identity of the substrates that are transported by both systems. GlcS binds in addition to glucose also the monosaccharides galactose and mannose (Albers et al. 1999; Elferink et al. 2001). These sugars are constituents of the glycosylation moieties of extracellular proteins from *S. solfataricus* (Elferink et al. 2001). Therefore, this transporter might not only be involved in uptake of sugars as a carbon source, but may also function in a "recycling" mechanism of the sugars that are released upon degradation of extracellular glycoproteins. This may explain why this system is constitutively expressed.

For three unlinked glycosyl hydrolases of *S. solfataricus*, α-glucosidase, β-glycosidase and α-amylase, it was previously shown that the addition of amino acids to the medium causes repression of hydrolase expression (Haseltine et al. 1999a). This suggests the presence of a global regulatory system that shuts down sugar metabolism, as soon as amino acids are present in the growth medium. Apparently, amino acids are the preferred carbon source to *S. solfataricus* rather than sugars. The arabinose transport system seems to be regulated by the same global regulator, as suggested for the hydrolases, whereas the glucose transport system appears invariant to the presence of amino acids. In some cases, the hydrolases do seem to respond differently to the amino acids. For example, glutamate has been described to be not repressive (Haseltine et al. 1999a), but with AraS (Fig. 7a) and AraV (Fig. 6), a strong repressive effect was observed with this amino acid in the medium. These differences may relate to strain differences, as the hydrolase studies were performed in *S. solfataricus* 98/2 (Haseltine et al. 1999a). The regulatory response to induction or repression of expression of the arabinose transporter seems equally fast. Upon addition of amino acids to the medium, the *araS* mRNA levels declined within 3 h (Fig. 7a) while the AraV protein levels (Fig. 6) were significantly reduced after 6 h. Similarly, addition of arabinose to tryptone-grown cells caused a doubling of the *araS* mRNA and AraV protein levels after 6 h. Our findings suggest that *S. solfataricus* prefers to utilize amino acids rather than arabinose. A similar phenomenon has been described for *Rhodospirillaceae*, *Rhizobium*, and enteric bacteria. These microorganisms prefer to use organic acids instead of sugars and this was termed "reverse catabolite repression" (Collier et al. 1996; Inui et al. 1996; O'Gara et al. 1989).

Currently, the mechanism of amino acid induced repression remains to be elucidated. Strikingly, the ATPase domains of both the glucose and arabinose transporters show a specific feature. The GlcV structure consists of an ATP-binding ABC domain and a *C*-terminal subdomain with an OB-like fold (Verdon et al. 2003). AraV has a similar domain organization as GlcV. The *C*-terminal subdomains show similarity to the regulatory domain of MalK, the ATPase of the maltose ABC transporter of *E. coli* (Chen et al. 2003) and *Thermococcus litoralis* (Bohm et al. 2002). In *E. coli*, the *C*-terminal subdomain has been shown to play a major role in regulation. In the absence of maltose, the regulatory domain binds MalT, the positive transcriptional regulator of the maltose transport operon (Panagiotidis et al. 1998). Due to this interaction, activation of the expression of the *mal* operon is prevented. However, in the presence of maltose, MalT is released from MalK whereupon expression of the *mal* operon is activated. This is redundant to the introduction. The *C*-terminal domains of GlcV and AraV may also fulfil a regulatory function in archaea, but to this date, such evidence is lacking. Expression of the maltose transporter of *T. litoralis* is influenced by the negative regulator TrmB (Lee et al. 2003). However, TrmB is not known to bind to MalK directly. In order to define the function of the *C*-terminal domain of GlcV and AraV, it will be essential to replace the genomic copy of either ATPase for a truncate that lacks the *C*-terminus. These studies will

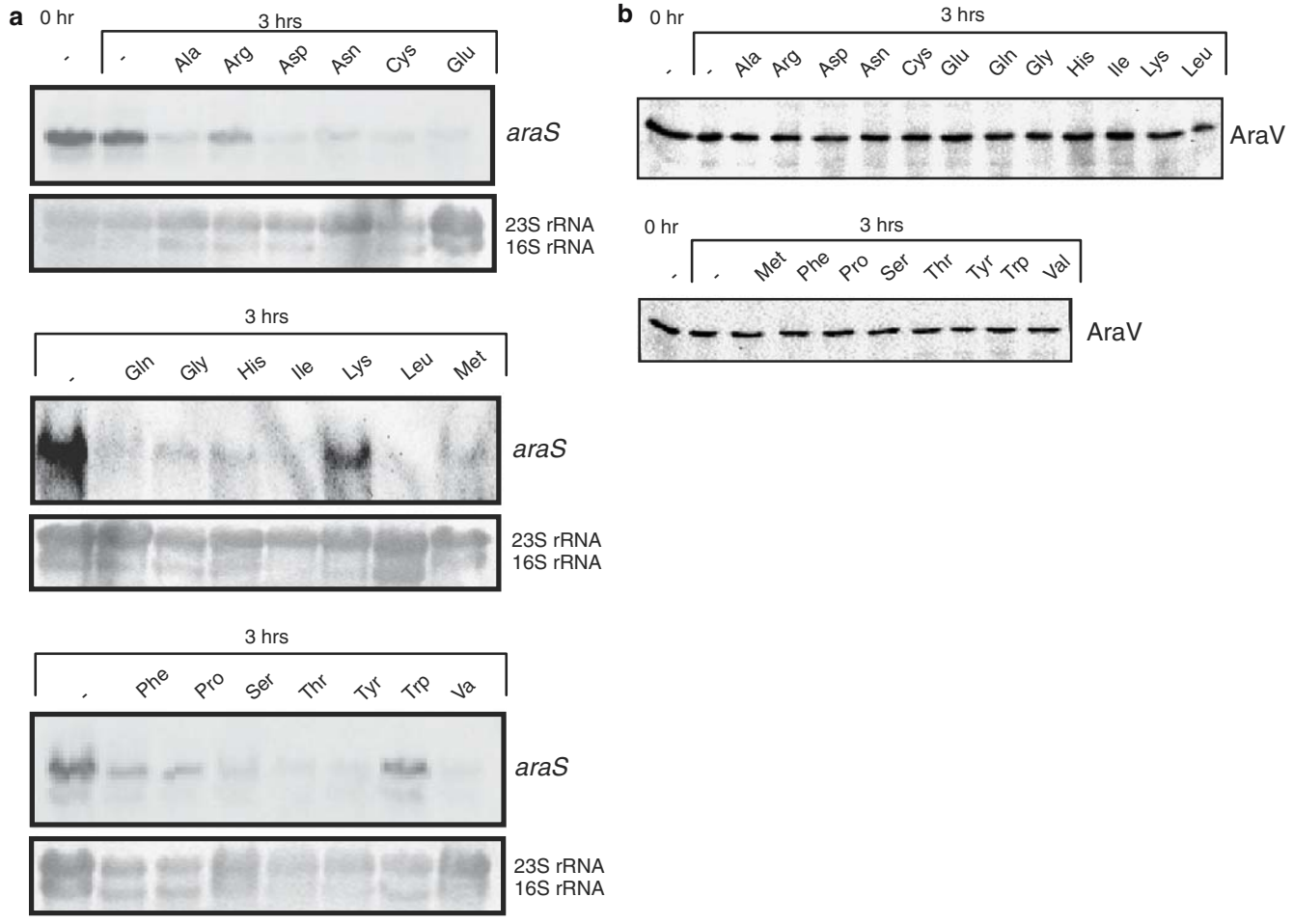

Fig. 7 Effect of amino acids on the *araS* mRNA **(a)** and AraV protein **(b)** levels in arabinose-grown *S. solfataricus* P2. **a** As a control for sample loading 23 and 16S rRNA levels were visualized on the same membrane by methylene blue staining before immunodetection (*lower panels*). Cells were pre-grown on arabinose and at OD_{600} of 0.4, the media were supplemented with the indicated amino acids at a 20 mM concentration. Growth was continued for another 3 h and the *araS* expression levels were determined by Northern blotting. As a control, total RNA was also isolated from control cells at time 0 and 3 h. **b** Protein expression levels of AraV in arabinose- grown *S. solfataricus* cells 3 h after addition of 20 mM of the indicated amino acids as described under (**a**)

reveal if the *C*-terminus of AraV plays a role in binding of a protein regulating an *ara* operon expression, as shown for MalK of *E. coli*.

In order to reveal the features of the regulatory networks that govern the expression of sugar transporters and metabolism, further studies in *S. solfataricus* are needed. At this stage, little is known about transcriptional regulation at the molecular level in extreme- and hyperthermophilic archaea. The recent developments in gene inactivation and homologous expression provide unique opportunities to study the physiological consequences of the regulatory network in *Sulfolobus* under relevant growth conditions. Future studies will focus on the identification of a transcriptional regulator controlling the expression of the arabinose transport genes.

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