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Novel Functional Aspects of the Membrane-Bound *exo*-Pyrophosphatase of the Hyperthermoacidophilic Archaeon *Sulfolobus* Are Provided by Analysis of Its Gene and the Adjacent Gene Cluster

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The gene of the previously described plasma-membrane-bound acidic pyrophosphatase (*exo*-PPase) and adjacent genes of the hyperthermoacidophilic crenarchaeon *Sulfolobus acidocaldarius* (DSM 639) were cloned and sequenced. The 4-kb gene cluster comprises four open reading frames (*sepp*, *simp*, *sabc*, and *satr*) encoding the pyrophosphatase, a small hydrophobic protein of unknown cellular function, a hydrophilic ABC transport ATPase, and an amino transferase. The four proteins have deduced molecular masses of 21, 16, 34, and 48 kDa, respectively. *Sepp*, *simp*, and *sabc* are transcribed as monocistronic mRNAs from which *sepp* and *sabc* have been heterologously expressed by in vitro translation using reticulocyte lysates. The *Sulfolobus acidocaldarius* acidic *exo*-pyrophosphatase is a membrane-residing protein anchored with five transmembrane α-helices. Alignments with protein sequences from databases together with predictions of membrane topology reveal a novel group of proteins with the conserved phosphatase motif KxxxxxRP-(x12-54)-PSGH-(x31-54)-SRxxxxxHxxxD. For none of them a phosphatase or pyrophosphatase acitivity has yet been described except for the authentic *Sulfolobus acidocaldarius* protein. On the basis of these investigations a direct role of the *exo*-PPase in dolichyl phosphate or pyrophosphate hydrolysis and in resistance to the peptide antibiotic bacitracin is discussed.

KEY WORDS: Acidic pyrophosphatase; PPase; phosphatase; plasma membrane; dolichyl phosphate; dolichyl pyrophosphate; glycosylation; bacitracin; ABC transporter; permease; archaea; *Sulfolobus acidocaldarius*.

INTRODUCTION

The crenarchaeon *Sulfolobus acidocaldarius* (DSM 639) is an extremely thermoacidophilic organism growing aerobically at pH 2.5 and 75°C (Brock *et al.*, 1972). Its plasma membrane is tightly connected to a very rigid surface layer composed of pseudo-crystalline glycoprotein arrays embedded in the tetraether-lipid-based matrix (Michel *et al.*, 1980). This overall membrane structure imposes an extraordinary high rigidity and pH stability to this organism ensuring survival under extreme conditions of temperature and acidity. However, less is known of the biochemical pathways leading to this functional archaeal membrane and therefore membrane biogenesis in archaea and glycosylation reactions at the archaeal cell surface are of growing interest.

In this respect a central key enzyme is the plasma-membrane-bound pyrophosphatase for which a dolichyl pyrophosphate hydrolyzing activity has been suggested (Meyer and Schäfer, 1992). Dolichyl phosphate is a key intermediate in *N*-glycosylation of eukaryal membrane proteins that has also been detected in archaea (Lechner and Wieland, 1989). In *Halobacterium salinarum* a C_{60}-dolichol is the carrier lipid of sulfated oligosaccharides containing glucuronic acids (Lechner *et al.*, 1985). The oligosaccharide is bound to this lipid via a monophosphate rather than a pyrophosphate bridge. The occurrence

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of dolichyl monophosphates and pyrophosphates turned out to depend on the first sugar moiety which is N-linked to specific Asn side chains in the polypeptide (Lechner and Wieland, 1989). While the dolichyl pyrophosphate hydrolysis in eukarya resides in the lumen of the endoplasmic reticulum, this reaction is likely to occur in archaea outside of the cytoplasmic compartment in the extracellular space where the transfer from dolichyl oligosaccharides takes place.

The plasma-membrane-bound inorganic pyrophosphatase of *Sulfolobus acidocaldarius* (*exo*-PPase) is oriented with its catalytic domains to the outside of the cells (Meyer and Schäfer, 1992). In its native, detergent solubilized state the enzyme forms a 70-kDa oligomer of undefined micellar composition. It differs in its broad substrate specificity, Mg^{2+} independence and the acidic pH optimum from the cytosolic localized soluble pyrophosphatase (Meyer *et al.*, 1995). The PP_i-cleaving activitiy restricted to membranes has been solubilized in a detergent-dependent procedure and could be assigned to a 18-kDa large membrane-residing protein which was strongly activated by specific tetraether lipid fractions. Its broad substrate specificity is restricted to phosphoanhydride bonds of different nucleoside tri- and diphosphates which have been optimally hydrolyzed at pH 3.5 and 75°C in the absence of monovalent and divalent cations. In contrast hydrolysis of phosphoester bonds in water-soluble phosphate compounds are not hydrolyzed at all. However, the proposed hydrolysis of dolichyl phosphate, pyrophosphate, or other isoprenoic phosphates by this *exo*-PPase has not been directly proven rather than concluded from inhibition by bacitracin. A homologous enzyme catalyzing the hydrolysis of inorganic pyrophosphate, ATP, and ADP has been characterized for the hyperthermoacidophilic *Sulfolobus solfataricus* strain 7 (Amano *et al.*, 1993).

The objectives of this study focus on (i) the identification of the gene, (ii) the genomic organization downstream of the gene, (iii) the detection of transcriptional units within this detected gene cluster, and (iv) bioinformatic approaches applied to corresponding polypeptides. Because of a similiar genomic structure in the gram-positive prokaryote *Bacillus licheniformis* encoding the bacitracin resistance operon an extended model with respect to the assumed dolichyl pyrophosphatase activity of the *S. acidocaldarius* exo-enzyme is discussed.

MATERIALS AND METHODS

Deduced from the sequence information of the N-terminal region of the *exo*-PPase protein a 90-bp large probe has been amplified with degenerated oligonucleotides P1 (5′ATG AG(A/G) AA(A/G) TA(C/T) TA(C/T) TA(C/T) TG3′) and P2 (5′CC(A/G/T) AT(A/G) TT(C/T) TG(C/T) TC(A/G/C/T) CC(A/G/C/T CC3′). The primers encode N-terminal and C-terminal parts of the 30 amino acid comprising N terminus of the *exo*-PPase protein as determined by Edman degradation (Meyer and Schäfer, 1992). The PCR was performed with 100 ng genomic *S. acidocaldarius* DNA, 2 units *Taq* DNA polymerase (MBI Fermentas), 2.5 mM $MgCl_2$, 200 μM dNTP, and 100 pmol P1 and P2 in a reaction volume of 100 μl. Amplification of the 90-bp PCR product was achieved in a touchdown mode in which the annealing temperature was adjusted from 50°C down to 35°C in a thermocycler with heated lid (Biometra). The correct sequence was verified by cloning and sequencing the 5′-phosphorylated PCR product in vector pBluescript II (KS+). Using the digoxigenin-labelled PCR probe in hybridization experiments with *S. acidocaldarius* genomic DNA, a 4-kb large EcoRI fragment was isolated. The fragment was ligated with EcoRI-restricted pBluescript KSII+ (Stratagene) and further cloned into *E. coli* XL1-blue. Plus and minus strand of the 4-kb insert were sequenced with a Liqor-sequencer (MWG Biotech) using the primer walking approach. The sequence of the 4-kb genomic DNA insert and the deduced open reading frames were deposited at the EMBL database with the accession number AJ550389.

Total RNA was extracted with Trizol reagent (Gibco) from *S. acidocaldarius* cells (DSM 639) freshly grown until an optical density (546 nm) of 0.3 O.D. RNA was electrophoresed on denaturing 1.5% (w/v) agarose gels containing 0.41 M formaldehyde in MOPS-buffer for 3 h. Afterwards gels were subjected to downward alkaline capillary blotting onto uncharged nylon membranes (Schleicher & Schüll) for 1 h at room temperature. Membranes were UV-cross-linked for 2 min, dried for 15 min at 80°C, and probed with mRNA-specific digoxigenin-labelled T3 RNA polymerase generated antisense in vitro transcripts after cloning corresponding genes into pCITE vectors (Novagen). Hybridizations were performed with 50 ng antisense *sepp* RNA/mL, 20 ng *simp* RNA/mL, and 50 ng *sabc* RNA/mL at 70°C. Chemiluminescence signals after hybridizations were detected as described elsewhere (Engler-Blum *et al.*, 1993; Schmitz *et al.*, 1991). In vitro translations of the pCITE cloned *sepp*, *simp*, and *sabc* genes with reticulocyte lysates in the presence of biotin-lysyl-tRNA were performed according to the manufacturer's instructions with 0.5 μg/μL plasmid template for 90 min at 30°C in 25-μL reaction volume (TNT coupled in vitro transcription/translation, Promega). Protein products of the in vitro translation were

subjected to SDS-PAGE and blotted onto polyvinylidene fluoride membranes for 1 h at room temperature in a semidry procedure. Biotinylated proteins by in vitro translation were detected with straptavidin-alkaline phosphatase conjugates and the chemiluminescence substrate CDP-Star (Roche) in the presence of Nitroblock II and Sapphire II enhancer (Tropix).

Sequence searches have been performed applying the search algorithm WU-BLAST to the database Swall with blossom 62 matrix at the EBI (Altschul *et al.*, 1997). Sequences were aligned with CLUSTAL W (Higgins *et al.*, 1994). For alignments sequences of the *Sulfolobus tokodaii* hypothetical protein ST 2226 (Q96YE4), *Sulfolobus solfataricus* hypothetical protein SSO 2653 (Q97VH2), *E. coli* YbjG protein (P75806), *Bacillus licheniformis* Bcr C protein (P42334), *Methanobacterium thermoautotrophicum* bacitracin related protein (O26889), *Bacillus subtilis* YwoA protein (P94571), and *Yersinia pestis* putative permease (Q8ZGH3) were extracted from the Swall database. Secondary structures were determined with the neural-network-based Predict Protein PHD program (Rost, 1996). Topology predictions of membrane proteins were done with the TMHHM program (Krogh *et al.*, 2001). Signal sequences were predicted according to Nielsen *et al.* (1997) and transfer enthalpies of polypeptides were calculated on the basis of the values given in Engelman *et al.* (1986).

RESULTS AND DISCUSSION

Genomic Organization of the 4-kb *S. acidocaldarius* DNA Fragment, Transcriptional and Translational Analysis

As shown in Fig. 1 the genomic 4-kb *S. acidocaldarius* EcoRI-DNA fragment is composed of four open reading frames which are not closely linked or overlapping. The first reading frame encompasses the *sepp* gene (nucleotide position 58-678) coding for the *exo*-PPase. The reverse complementary strand of the second open reading frame *simp* encodes a hydrophobic protein of yet unknown function (complement of position 759-1208). In contrast the third open reading frame *sabc* contains the genetic information of an ABC transport ATPase of unknown physiological function (position 1417-2329). The fourth frame at the 3′-end of the sequenced genome fragment harbors the *satr* gene of a group III pyridoxalphosphate-dependent amino transferase (position 2590-3878). Archaeal transcription promoter sequences of box A- and box B-type (Thomm, 1999; Soppa, 1999) are present in position −29 and +1 respective to the ATG start codon of the *sepp* gene. However, TATA-alike consensus sequences are obviously lacking for the other open reading frames of this 4-kb gene cluster. Furthermore, for neither of these genes classical archaeal Shine-Dalgarno homologous consensus sequences (Tolstrup *et al.*, 2000) and T-rich transcription termination sequences (Reiter *et al.*, 1988) have been found.

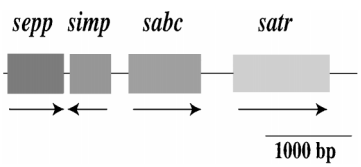

Fig. 1. Schematic representation of the *S. acidocaldarius* genomic 4-kb EcoRI DNA fragment encoding the genes *sepp*, *simp*, *sabc*, and *satr*. Arrows from left to right indicate the upper 5′ → 3′ coding strand. The arrow in the opposite direction indicates the coding strand as complementary to the upper strand. Numbers indicate calculated molecular masses of the gene products.

Northern blot analysis using homologous RNA probes derived from in vitro transcription of the corresponding genes demonstrate that the first three genes were transcribed under the heterotrophic growth conditions of *S. acidocaldarius*. In addition, mRNA of different length for *sepp*, *simp* and *sabc* (Fig. 2(A).) have been detected. Limited by the resolving strength of the gel system the lengths of the two *sepp*- and *simp*-transcripts could not be distinguished. However, the different transcripts detected in the cellular *S. acidocaldarius* RNA argue for monocistronic messages. Transcriptional analysis of the *satr* gene was not performed here.

Translation competent reticulocyte lysates were further used to visualize protein products of *sepp*, *simp*, and *sabc*. Using in vitro transcribed *sepp*- and *sabc*-mRNA from the coding regions of pCITE-*sepp* and pCITE-*sabc* vectors two different polypeptides of approximately 18 kD and 34 kD were expressed by in vitro translation (Fig. 2(B)). The size of the two polypeptides agree approximately with the computed molecular mass obtained from the nucleotide sequence data. While expression of *sepp*- and *sabc*-mRNA could be unambiguously shown, the expression of *simp* cloned into the pCITE plasmid failed (data not shown). Because of the strong hydrophobicity of the simp polypeptide, higher-molecular-weight products may lead to aggregation and may thus escape detection in SDS gel electrophoresis and protein transfer. Actually, for the same reason the in vitro-expressed hydrophobic *exo*-PPase protein was not detected when high amounts of the in vitro-translation product were loaded onto the SDS gel.

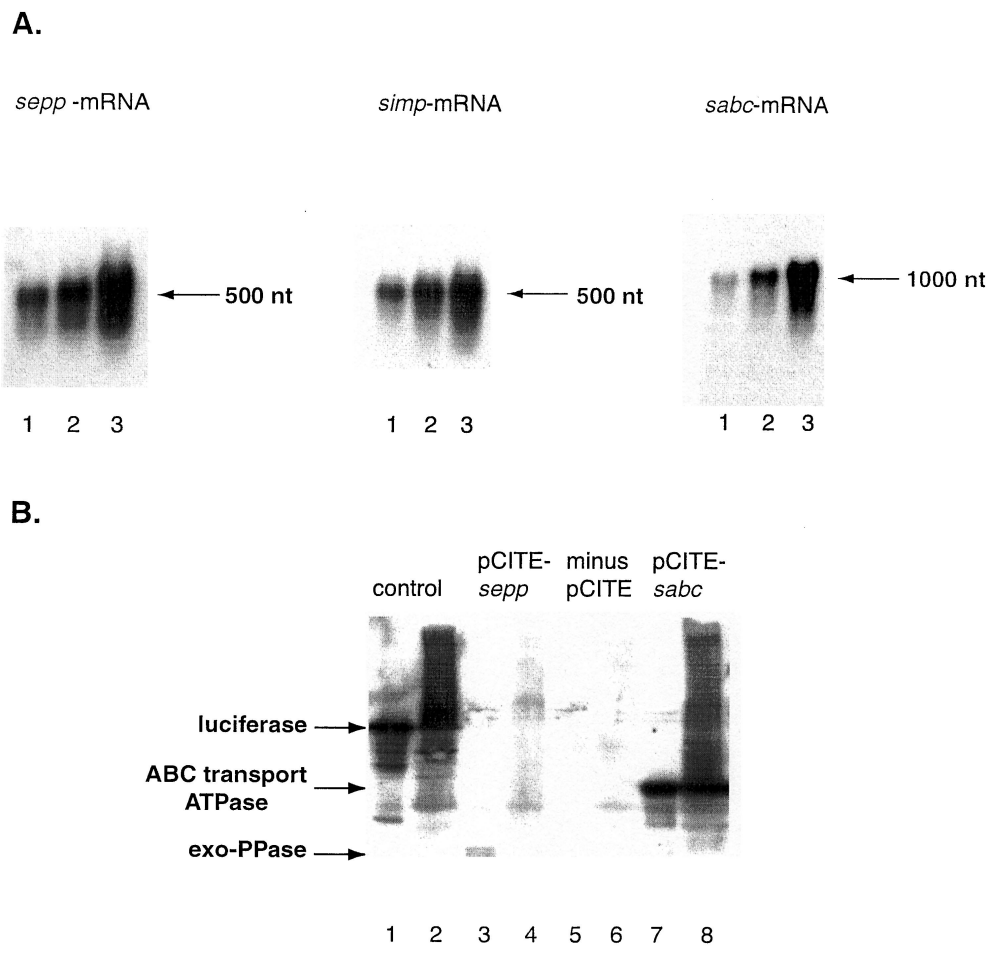

Fig. 2. (A) Northern analysis of *sepp*-, *simp*-, and *sabc*-mRNA with full-length antisense in vitro transcripts. Lanes 1, 2 and 3: 10, 20, and 50 μg *S. acidocaldarius* cellular RNA was applied to denaturing agarose and subsequently analyzed by Northern blotting. The size of the mRNAs on Northern blots were estimated by means of a size standard ranging from 500 to 6000 ribonucleotides. (B) In vitro transcription/translation (ivT/T) of *sepp* and *sabc* genes. Lanes 1 and 2: ivt/T samples with control plasmid containing luciferase gene; Lanes 3 and 4: ivT/T samples with pCITE-*sepp* plasmid; Lanes 5 and 6: ivT/T samples without any plasmid; Lanes 7 and 8: ivT/T samples with pCITE-*sabc* plasmid. 1 μL (Lanes 1, 3, 5, 7) and 5 μL (Lanes 2, 4, 6, 8) of ivT/T assay was subjected to SDS-PAGE.

Hydrophobicity, Secondary Structure, and Topology Predictions

The *exo*-PPase as deduced from the *sepp* nucleotide sequence turns out to be a basic and membrane-bound protein with a high amount of hydrophobic residues and a calculated molecular mass of 21 kDa. Secondary structure predictions reveal a content of 72.3% α-helix, 7.3% β-sheet and 20.4% random coil. The calculated transfer enthalpy of the protein is displayed in Fig. 3 and obviously displays four hydrophobic segments including the hydrophobic core of the N-terminal localized signal peptide. Since the signal peptide sequence was found in the isolated mature protein (Meyer and Schäfer, 1992), this first transmembrane domain is assumed to anchor the protein together with other hydrophobic domains in the tetraether-lipid-based plasma membrane of *S. acidocaldarius* cells. Its strong interaction with the plasma membrane in vivo also explains the dependence of detergent solubilized pyrophosphatase activity on the presence of tetraether lipids (Meyer and Schäfer, 1992). As shown in Fig. 3 also the 16-kDa simp-protein resides with its hydrophobic N-terminal and C-terminal end in the membrane. The N-terminal domain of the simp protein encompasses a signal peptide-alike structure as found for the *exo*-PPase. In contrast, the 34-kDa ABC transport ATPase encoded by *sabc* appears

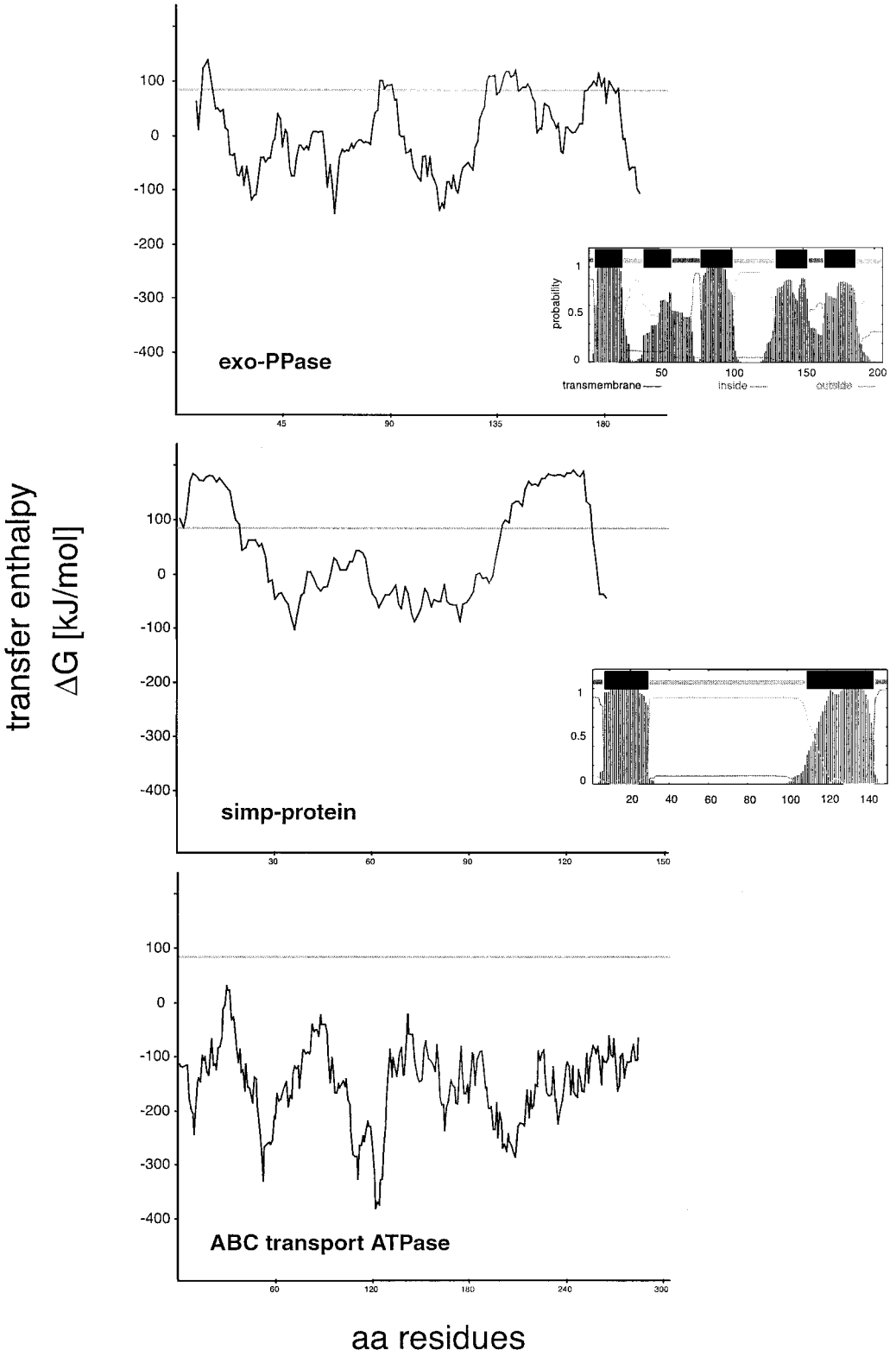

Fig. 3. Hydropathy profiles of *S. acidocaldarius exo*-PPase, simp-protein, and the ABC transport ATPase. Inserts: transmembrane helices and predicted loop Orientations.

to be a hydrophilic protein with a strong negative transfer enthalpy and no apparent transmembrane domains.

Topology calculations suggest five transmembrane α-helices in the *S. acidocaldarius exo*-PPase in agreement with its high α-helical content. Interhelical regions of the membrane-incorporated protein are distributed according to the "positive-inside" rule (Andersson and von Heijne, 1994). In this context a strong positive charge cluster of the first and second inside localized region may configure the *exo*-PPase in a correct position with respect to the plasma membrane. Interestingly, the functional residues of the catalytic domain are predicted as oriented outwards (Fig. 4). Also the simp protein comprises two membrane-spanning domains, one in the N-terminal protein region

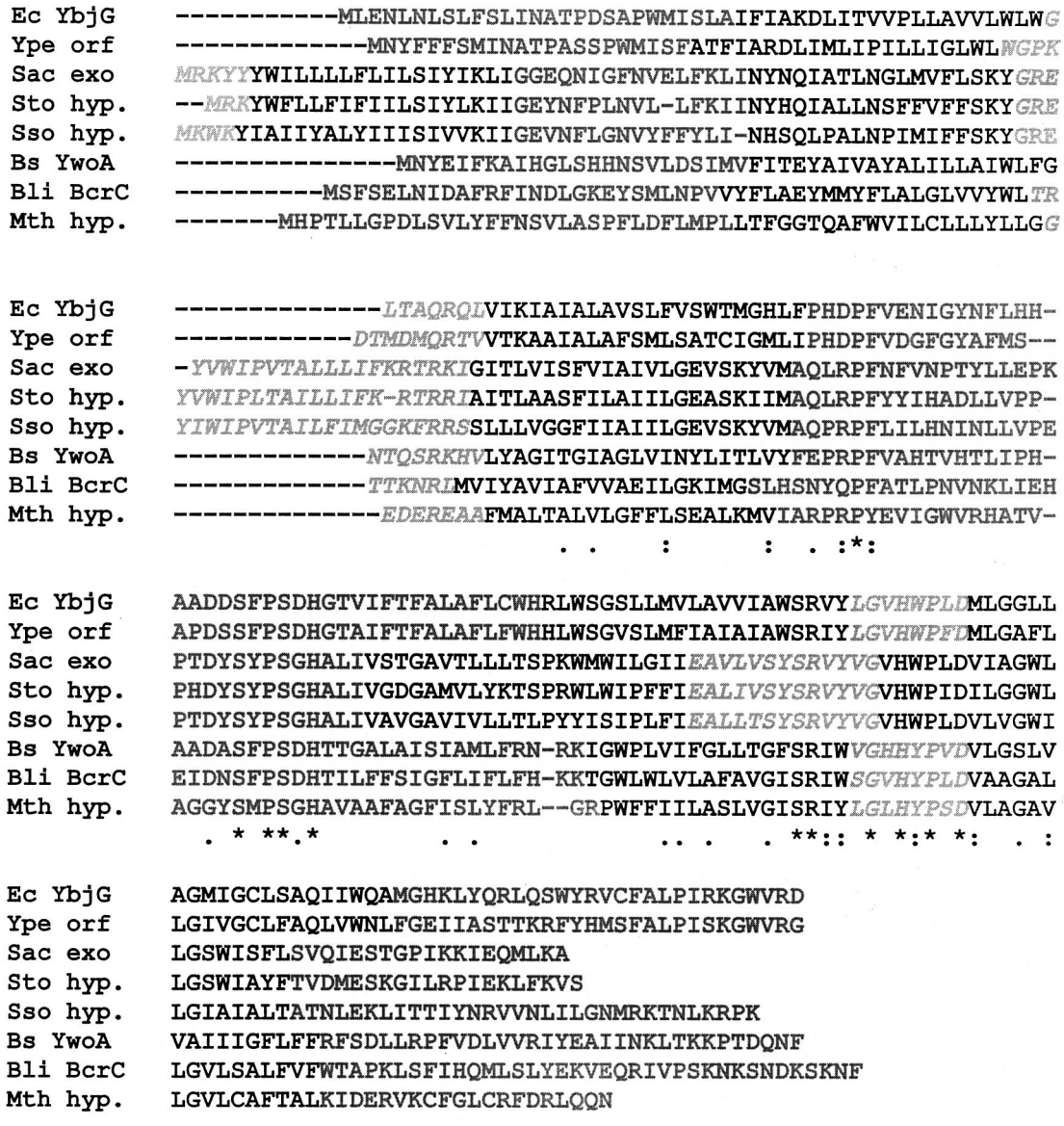

Fig. 4. Sequence alignment and membrane topology prediction of *S. acidocaldarius exo*-PPase with homologous archaeal and bacterial proteins. *E. coli* (Ec) YbjG, *Yersinia pestis* (Ype) hypothetical (hyp.) protein, *Sulfolobus tokodaii* (Sto), hypothetical protein, *Sulfolobus solfataricus* (Sso) hypothetical protein, *Bacillus subtilis* (Bs) YwoA, *Bacillus licheniformis* (Bli) BcrC, and *Methanobacterium thermoautotrophicum* (Mth) hypothetical protein. The different grey scales correspond to the calculated membrane topology of the residues. Pale grey italic – sequence regions to be predicted inside; black – transmembrane α-helices; grey – sequence regions to be predicted outside.

and the other at the C terminus. The intervening sequence between both membrane domains is predicted being outside. In contrast, the ABC transport ATPase sequence does not possess transmembrane helices. In fact, many ATP hydrolyzing proteins are components of larger membrane integral permease complexes orientated with their catalytic active centers to the cytoplasmic compartment (Driessen *et al.*, 2000; Higgins, 1992).

The *S. acidocaldarius exo*-Pyrophosphatase Belongs to a Novel Protein Family of Membrane-Residing Phosphatases

The *S. acidocaldarius exo*-PPase protein displays distinct sequence similarity to the *E. coli* YbjG/*B. subtilis* YwoA/*B. licheniformis* Bcr C protein family (Fig. 4). Sequence searches together with detailed topology predictions extend this family now to hypothetical proteins of *S. tokodaii*, *S. solfataricus*, *M. thermoautotrophicum*, and *Y. pestis*. The sequence similarity is highest among the crenarchaeal sequences (51–63%), but decreases to 17% when crenarchaeal and bacterial sequences are compared. Remarkably the similarity among the crenarchaeal sequences to the *M. thermoautotrophicum* protein (23%) is as low as to the bacterial members. In contrast, a high similarity was also obtained between the gram-negative *E. coli* YbjG and *Y. pestis* hypothetical protein (57%) suggesting a homologous cellular function of both. All proteins of this class range in molecular mass from 21 to 25 kDa and are more or less basic proteins (pI(*E. coli* YbjG) = 7.9, pI(*S. solfataricus*) = 9.1).

Despite the low sequence similarity, four transmembrane α-helices in the bacterial/euryarchaeal species and five in the crenarchaeal ones could be deduced from topology predictions (Fig. 4). Thus, all members are clearly classified as integral membrane proteins. The transmembrane topology of the three crenarchaeal sequences differs from the bacterial and euryarchaeal species as their N-terminal domains contain an additional signal sequence-alike structure. Since the signal sequence is present in the mature *S. acidocaldarius exo*-PPase as mentioned above, the corresponding mature proteins of *S. tokodaii* and *S. solfataricus* are likely to carry also this signalling domain important for protein translocation.

The cellular function of this novel protein class shown here is unknown. At least for *B. licheniformis* BcrC and *B. subtilis* YwoA in cooperation with the Bcr-A and B proteins a bacitracin insensitivity conferring activity has been reported (Ohki *et al.*, 2003; Podlesek *et al.*, 1995). Remarkably, both the *B. licheniformis* Bcr C and *B. subtilis* YwoA were not described as pyrophosphatases which, in contrast, was clearly demonstrated for the corresponding purified *S. acidocaldarius* protein (Meyer and Schäfer, 1992). Moreover, the sequence alignment (Fig. 4) umambigiously unravels a conserved sequence motif which was previously identified as a tripartite phosphatase motif essential for hydrolysis of phosphate compounds (Stukey and Carman, 1997). The motif encompasses the residues KxxxxxRP-(x12-54)-PSGH-(x31-54)-SRxxxxxHxxxD. This sequence is shared by several membrane integral lipid phosphatases, like the Mg^{2+}-independent phosphatidic acid phosphatases of the PAP2-family, the mammalian glucose-6-phosphatase of the endoplasmic reticulum and a collection of bacterial nonspecific acid phosphatases. Strikingly, the same motif was detected in yeast proteins YDR 503C (Lpp1p) and YDR 284C (Dpp1p) which both possess an Mg^{2+}-independent hydrolytic activity against dolichyl phosphate, dolichyl pyrophosphate and other isoprenoid phosphates/pyrophosphates (Faulkner *et al.*, 1999). Here, this phosphatase motif is strongly conserved in the three archaeal proteins. Deviating from this motif K is lacking in the first phosphatase domain in all other members and G in the second signature domain is substituted by D. However, the similiar arrangement of catalytic important residues probably specifies the active site of this membrane integral phosphatase/pyrophosphatase class. Obviously, most of these residues are positioned on an outside-oriented interhelical loop.

Structural Motifs of the *S. acidocaldarius* ABC Transport ATPase and the Simp Protein

The *sabc* gene encodes a 34-kDa large protein which obviously does not contain transmembrane α-helices as shown above for the *exo*-pyrophosphatase. It displays significant similaritiy to many other multidrug resistance conferrring ATP-binding cassette transport ATPases (here not shown). Most of these proteins are detected by genome sequencing. However, some of them like the *B. licheniformis* Bcr A protein are involved in resistance to antibiotics. In agreement the *S. acidocaldarius* ABC transport ATPase has two conserved sequence motifs which are essential for ATP-binding and -hydrolysis: the Walker A motif (G/AxxGxNGA/SGKT/ST) in position 28-31 and the Walker B motif (DEP/VxxGLD) in position 158-165 (Higgins, 1992). Actually, the highly conserved regions of this *S. acidocaldarius* ATPase can be superimposed with the known tertiary structure of the *Salmonella* HisP protein (Hung *et al.*, 1998) (not shown here). Other well-conserved residues are present in the *S. acidocaldarius* sequence (K10, G57, G63, S135, G137, P152, G209). In contrast the C-terminal stretches within

this protein class exhibit low similarity of length and sequence.

In contrast to the *exo*-pyrophosphatase and to the ABC ATPase no apparent signature can be derived for the simp protein. However, homologous-membrane-embedded proteins containing transmembrane helices both, at the N and the C terminus, can also be deduced from genomes of *S. tokodaii* and *S. solfataricus*. The *S. acidocaldarius* simp protein (151 residues), *S. tokodaii* ST 1169 protein (160 residues), and *S. solfataricus* hypothetical protein SSO 2083 (167 residues) offer a low sequence similarity between 19 and 28% with most of the conserved residues in the respective transmembrane sections.

CONCLUSIONS AND PERSPECTIVES

Structural evidences demonstrated in this study favor a previously presented model (Meyer and Schäfer, 1992) in which the *S. acidocaldarius exo*-pyrophosphatase catalyzes the hydrolysis of dolichyl phosphate/pyrophosphate or related membrane-bound isoprenoid compounds. Supporting this view dolichyl-related metabolites were detected in archaea and a dolichyl phosphate cycle homologous to the eukaryal one was previously proposed to glycosylate surface layer proteins (Lechner and Wieland, 1989). Inferred from its membrane topology the *exo*-PPase may hydrolyze in vivo-membrane-bound isoprenoid phosphates or diphosphates, but not soluble diphosphate containing substrates as previously determined by in vitro assays. Now a direct hint to unravel experimentally catalytic important residues of the *S. acidocaldarius exo*-PPase in dolichyl phosphate/pyrophosphate hydrolysis could be given. Moreover, it is tempting to propose also for the other members of the newly defined phosphatase family to hydrolyze phosphate or pyrophoshate containing lipids which remains to be shown experimentally.

Another appealing but hypothetical aspect is a function of the whole *sepp–simp–sabc* gene cluster as a device oviding bacitracin resistance. Bacitracin interacts with substrate loaded isoprenoid pyrophosphatases (Storm, 1974); thus, the latter may serve as sensors or binding proteins. If it applies that like in *B. licheniformis* or *B. subtilis* (Ohki *et al.*, 2003; Podlesek *et al.*, 1995) helper proteins with ABC-transporter homology are involved, the above genes may encode also an antibiotic defense system. That is supported by a significant relation of the *sabc*-gene product to the respective Bcr-A protein of *B. subtilis*. Moreover, at pH 2.5, *S. acidocaldarius* is indeed resistant to bacitracin, but not at pH 5.5 which is far above the pH optimum of the bacitracin binding sepp-PPase. In addition, under the latter conditions cellular ATP is too low in *S. acidocaldarius* (Moll and Schäfer, 1988) to drive the postulated ABC transporter-linked bacitracin-permease.

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REFERENCES

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997). *Nucleic Acids Res.* **25**, 389–402.
Amano, T., Wakagi, T., and Oshima, T. (1993). *J. Biochem. (Tokyo)* **114**, 329–333.
Andersson, H., and von Heijne, G. (1994). *EMBO J.* **13**, 2267–2272.
Brock, T. D., Brock, K. M., Belly, R. T., and Weiss, R. L. (1972). *Arch. Microbiol.* **84**, 54–68.
Driessen, A. J. M., Rosen, B. P., and Konings, W. N. (2000). *Trends Biochem. Sci.* **25**, 397–401.
Engelman, D. M., Steitz, T. A., Goldman, A. (1986). *Annu. Rev. Biophys. Biophys Chem.* **15**, 321–53.
Engler-Blum, G., Meier, M., and Muller, G. A. (1993). *Anal. Biochem.* **210**, 235–244.
Faulkner, A., Chen, X., Rush, J., Horazdovsky, B., Waechter, C. J., Carman, G. M., and Sternweis, P. C. (1999). *J. Biol. Chem.* **274**, 14831–14837.
Higgins, C. F. (1992). *Annu. Rev. Cell Biol.* **8**, 67–113.
Higgins, D., Thompson J., Gibson, T., Thompson, J. D., Higgins, D. G., and Gibson T. J. (1994). *Nucleic Acids Res.* **22**, 4673–4680.
Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E. L. L. (2001). *J. Mol. Biol.* **305**, 567–580.
Hung, L.-W., Wang, I. X., Nikaido, K., Liu, P.-Q., Ferro-Luzzi Ames, G., and Kim, S.-H. (1998). *Nature* **396**, 703–707.
Lechner, J., Wielund, F., and Sewpes, M. (1985). *J. Biol. Chem.* **260**, 8984–8989.
Lechner, J., and Wieland, F. (1989). *Annu. Rev. Biochem.* **58**, 173–194.
Meyer, W., and Schäfer, G. (1992). *Eur. J. Biochem.* **207**, 741–746.
Meyer, W., Moll, R., Kath, T., and Schäfer, G. (1995). *Arch. Biochem. Biophys.* **319**, 149–156.
Michel, H., Neugebauer, D.-C., and Oesterhelt, D. (1980). In *Electron microscopy at molecular dimension* (Baumeister, W., and Vogel, W., eds.), Springer, Berlin, pp. 27–35.
Moll, R., and Schäfer, G. (1988). *FEBS Lett.* **232**, 359–363.
Nielsen, H., Engelbrecht, J., Brunak, S., and von Heijne, G. (1997). *Protein Eng.* **10**, 1–6.
Ohki, R., Tateno, K., Okada, Y., Okajima, H., Asai, K., Sadaie, Y., Murata, M., and Aiso, T. (2003). *J. Bacteriol.* **185**, 51–59.
Podlesek, Z., Comino, A., Herzog-Velikonja, B., Zgur-Bertok, D., Komel, R., and Grabnar, M. (1995). *Mol. Microbiol.* **16**, 969–976.
Reiter, W. D., Palm, P., and Zillig, W. (1988). *Nucleic Acids Res.* **16**, 2445–2459.
Rost, B. (1996). *Meth. Enzymol.* **266**, 525–539.
Schmitz, G. G., Walter, T., Seibl, R., and Kessler. C. (1991). *Anal. Biochem.* **192**, 222–231.
Soppa, J. (1999). *Mol. Microbiol.* **31**, 1589–1601.
Storm, D. R. (1974). *Ann. N.Y. Acad. Sci.* **235**, 387–398.
Stukey, J., and Carman, G. M. (1997). *Protein Sci.* **6**, 469–472.
Tolstrap, N., Sonsen, C. W., Garrett, R. A., and Clausen, J. G. (2000). *Extremophiles* **4**, 175–179.
Thomm, M. (1999). *FEMS Microbiol. Rev.* **18**, 159–171.