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Membrane-bound ATPase of a thermoacidophilic archaebacterium, *Sulfolobus acidocaldarius*

Takayoshi Wakagi *,** and Tairo Oshima **

Laboratory of Natural Products Chemistry, Tokyo Institute of Technology, Yokohama, and the Laboratory of Biochemical Reactions and Biocatalyst, Mitsubishi-Kasei Institute of Life Sciences, Tokyo (Japan)

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The membranes of *Sulfolobus*, a thermoacidophilic archaebacterium showed two types of ATP hydrolyzing activity. One was that of a neutral ATPase at an optimum pH around 6.5. This enzyme was activated by 10 mM sulfate with a shift of optimum pH to 5. In these respects, the enzyme was similar to membrane-bound ATPase of *Thermoplasma*, another thermoacidophilic archaebacterium, reported by Searcy and Whatley ((1982) Zbl. Bakt. Hyg., I. Abt. Orig. C3, 245–257). The enzyme hydrolyzed ATP and other NTPs, but not ADP or AMP. It was highly thermostable, but irreversibly inactivated in 0.1 M HCl. The other activity was that of an acidic apyrase at an optimum pH around 2.5. This enzyme was extremely stable toward high temperature and acid and inhibited by sulfate. Both of these ATP hydrolyzing enzymes were resistant to *N,N'*-dicyclohexylcarbodiimide (DCCD), azide, oligomycin, *N'*-ethylmaleimide, *p*-chloromercuribenzoate, orthovanadate, or ouabain. *Sulfolobus* ATPases differ from F_1 and other transport ATPases so far described.

Introduction

Sulfolobus acidocaldarius, a thermoacidophilic archaebacterium found in hot-acid springs, grows optimally at pH 2–3, 70°C [1]. The organism is a facultative chemoautotroph oxidizing elemental sulfur to sulfate [1,2] and can grow heterotrophically on nutrient medium [1]. Although *Sulfolobus* resembles *Thiobacillus* in its sulfur metabolism, it was classed as archaebacteria since it contains characteristic 16S rRNA [3] and its membrane lipid is the glycerol-ether type [4,5].

Archaebacteria are of interest from the stand point of the possible position they occupy in early biological evolution. In recent years, thermoacidophilic, methanogenic, halophilic and anaerobic thermophilic archaebacteria have been extensively studied. The results of certain studies suggest that the organism may have derived directly from a primitive life [6]. Still other studies provide an attractive hypothesis that, phylogenically, they are related more to eukaryote than to other prokaryotes, and possibly to primitive eukaryotes [7–10]. Thus, the phylogeny of these archaebacteria is a topic of current studies.

Membrane-bound ATPase is of interest for its evolutional biochemical significance and energy transduction [11] of archaebacteria. The membrane ATPase of *Methanobacterium* has been found to resemble eubacterial ATPase (F_0F_1)

* To whom correspondence should be addressed.
** Present address of both authors: The Laboratory of Natural Products Chemistry, Tokyo Institute of Technology, Nagatsuta, Yokohama, Kanagawa 227, Japan.

Abbreviations: DCCD, *N,N'*-dicyclohexylcarbodiimide; Pipes, 1,4-piperazinediethanesulfonic acid.

[12–14]. Searcy and Whatley [15] reported the membrane ATPase of *Thermoplasma*, a thermoacidophilic archaebacterium, to be sulfate dependent and different from F_0F_1, $(Na^+ + K^+)$-ATPase, and Ca^{2+}-ATPase.

In the present study, the *Sulfolobus* membrane was found to have an ATPase quite similar to that in *Thermoplasma*. Another ATP-hydrolyzing enzyme in the *Sulfolobus* membrane occurred at an optimal pH around 2.5. Characterization of these two (acid and neutral) ATP-hydrolyzing enzymes in *Sulfolobus* membrane is presented.

Materials and Methods

Culture

The thermoacidophilic archaebacterium in this study was isolated from an acidic spa in Beppu Hot Springs, Japan. The isolate was able to grow autotrophically on elemental sulfur. The isolate lacked a peptide-glycan cell wall, but had a proteinacious outer layer [16]. The membrane consisted of C_{40}-tetraether lipids. The isolate was named *Sulfolobus acidocaldarius* strain 7.

The organism was grown at 75°C, as described in Ref. 16. Cells were collected and washed with 50 mM NaCl until the washings was colorless and were stored frozen at −20°C.

Preparation of cellular fractions

The frozen cells were thawed and incubated in 50 mM Tris-HCl (pH 7.5) with a small amount of DNAase and RNAase, at 37°C for 1 h. This was followed by a second freezing and thawing. The cell suspension was centrifuged at $5000 \times g$ for 10 min. The resulting heavily turbid supernatant was further centrifuged at $15000 \times g$ for 60 min. The pellet thus obtained was suspended in 50 mM Tris-HCl and centrifuged at $15000 \times g$ for 60 min. This procedure was repeated several times until the supernatant was colorless. The supernatant and pellet from the $15000 \times g$ centrifugation were designated S_0 and P_0, respectively. P_0 was washed with 0.5 M KCl by centrifugation several times until its washings were nearly free from protein. The resultant supernatant and pellet were desinated P_1 and S_1, respectively. S_0 and S_1 were dialyzed against 25 mM Tris-HCl (pH 7.0). P_0 and P_1 were washed with distilled water. All the cellular fractions obtained were stored frozen at −20°C without change in their biochemical characteristics except that small amounts of ATPase became detached from P_0 by repeated freeze-thawings.

Electron microscopy

The samples were fixed with 1% glutaraldehyde, postfixed in OsO_4, dehydrated in ethanol, and embedded in epoxy resin. Sections were stained with uranyl acetate and lead nitrate.

P_i determination

P_i was determined either by the method of Lin and Morales [17] or that of Rathbun and Betlach [18].

Enzymatic activity

Hydrolysis of ATP or other nucleotides were measured by the release of P_i. The standard assay mixture contained in a final volume of 100 μl, 2 mM ATP (or other nucleotide), 1 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.0 at 55°C), and the enzyme sample. After 20 min at 55°C, the reaction was terminated by cooling on ice, followed by the immediate addition of the reagents for P_i determination. Certain chemicals were also added to the assay mixture if necessary. The inorganic pyrophosphatase activity was assayed in a reaction mixture containing 50 mM Tris-HCl (pH 7.0), 2 mM PP_i, 2 mM $MgCl_2$, and the enzyme sample. The P_i released at 55°C during the 20 min period was determined. The activity was expressed as P_i produced under the experimental conditions. NADH oxidase activity was determined spectrophotometrically by following the absorbance at 340 nm [19] at 37°C. The assay mixture contained 0.2 mM NADH, 50 mM Tris-HCl (pH 7.0), and the enzyme sample. *p*-Nitrophenylphosphatase activity was determined spectrophotometrically by following the absorbance at 400 nm [20] at 37°C. The assay mixture contained 10 mM *p*-nitrophenyl phosphate, 2 mM $MgCl_2$, 50 mM Tris-HCl (pH 7.0) and a suitable amount of the enzyme preparation. Pyruvate kinase activity was measured by the coupling method as follows. The reaction mixture contained 20 mM Tris-HCl (pH 7.0), 2 mM phospho*enol*pyruvate, 2 mM ADP, 20 mM KCl, and the sample enzyme. Following incubation for 10 min at 55°C, the mixture was cooled on ice, and

centrifuged immediately. The resultant supernatant was mixed with an equal volume of buffer containing 20 mM Tris-HCl (pH 7.0), 0.4 mM NADH and 40 μg/ml lacatate dehydrogenase (Boehringer). The decrease in absorbance at 340 nm was recorded. From this value, the amount of lactate produced was calculated. Glucose-6-phosphatase and fructosebisphosphatase activities were determined by P_i release following incubation of the enzyme preparation with 2 mM of either glucose 6-phosphate or fructose bisphosphate in 50 mM Tris-HCl buffer (pH 7.0) at 55°C for 20 min.

Nucleotide analysis

A reaction mixture containing 1.8–2 mM of the total nucleotides was diluted 100-fold with ice-cold water and centrifuged immediately to remove the membranes. The resultant supernatant (10–20 μl) was analyzed by reverse phase high performance liquid chromatography.

The amounts of nonenzymatically produced ADP, AMP and P_i from ATP were estimated in the control experiments, and subtracted from the corresponding values obtained in the sample experiment.

Protein determination

Protein was determined by the method of Wang and Smith [21].

Results

Enzymatic activity of the cellular fractions

The amount of a typical cytoplasmic enzyme, pyruvate kinase, was negligible in P_0, as evidenced from Table I. Electron microscopy indicated P_0 was free of cytosol (Fig. 1). Thus, P_0 is a practically homogeneous cell envelope fraction not contaminated by cytosol. In contrast to *p*-nitrophenylphosphatase, NADH oxidase and ATPase activities were associated primarily with the membrane fraction P_0, but most of these activities were solubilized when P_0 was washed with 0.5 M KCl. Although the final membrane fraction P_1 had an ATPase activity less than that of the other fractions, the possibility that the activity is due to contamination of S_1 can be ruled out by several lines of evidence; the activity in S_1 was completely lost at pH 4.5, while that in P_1 was significant (Fig. 2); the activity in S_1 and P_1 was inhibited by 2 and 75%, respectively, by 1 M urea. Further washing of P_1 with 0.5 M KCl caused no release of ATPase. These observations indicate the ATPase in P_1 to differ from that in S_1 and to be membrane-bound. In all the cellular fractions, the inorganic pyrophosphatase activity was noticeably higher than the other enzyme activities tested.

Hydrolysis of various substrates

The ability of four cellular fractions, S_0, P_0, S_1 and P_1, to hydrolyze various nucleotides, sugar

TABLE I

ENZYMATIC ACTIVITY OF THE *SULFOLOBUS* FRACTIONS

Fractions S_0 and P_0 correspond to the cytosol and crude membrane fractions, respectively. P_0 was further washed with 0.5 M KCl to obtain the S_1 soluble fraction and the P_1 membrane fraction. The relative distribution of protein in each fraction is indicated as the amount of protein in the whole cell being 100%. PPase, pyrophosphatase; NADH ox., NADH oxidase; pNPPase, *p*-nitrophenylphosphatase.

Fractions	Protein (%)	Specific activity (nmol P_i/min per mg) of				
		ATPase	pNPPase	PPase	NADH ox.	pyruvate kinase
S_0	48	16	44	2400	0.60	8.7
P_0	52	25	0.57	600	1.9	0.0
S_1	16	69	2.2	700	0.96	–
P_1	36	9.5	0.14	105	0.19	–

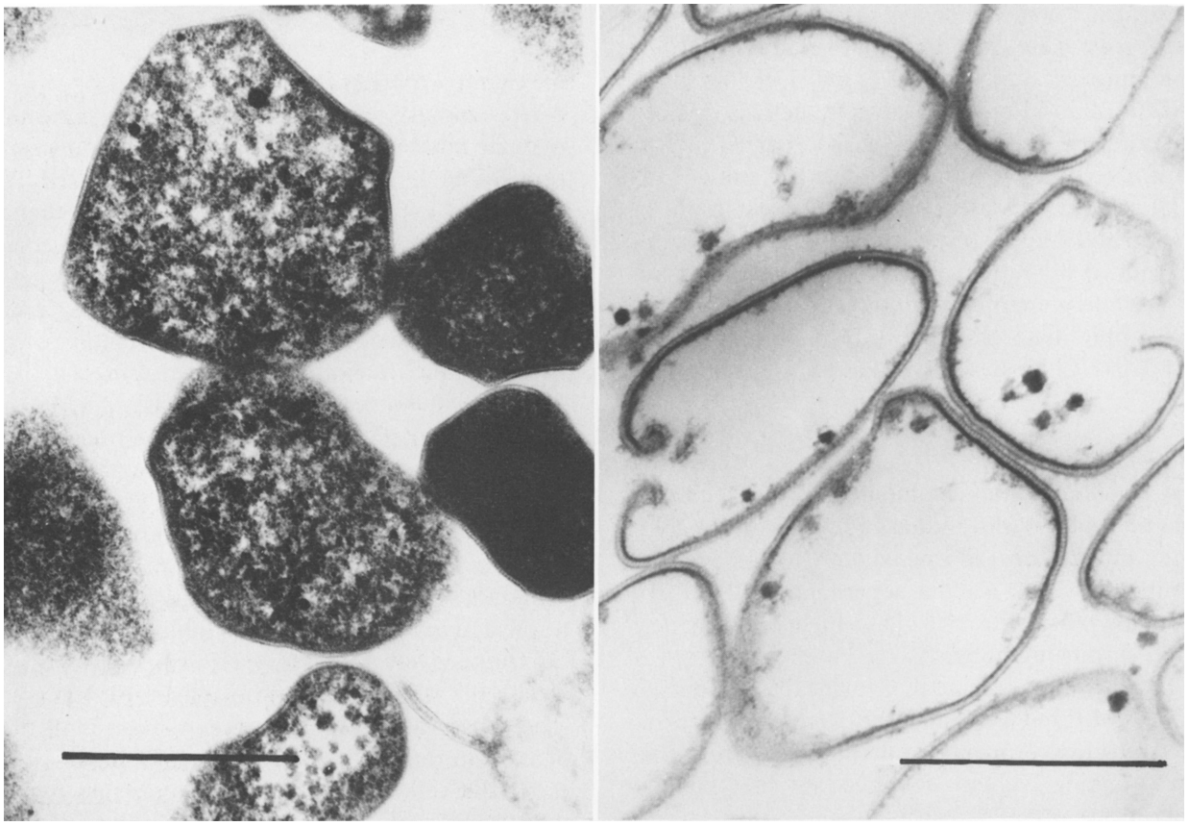
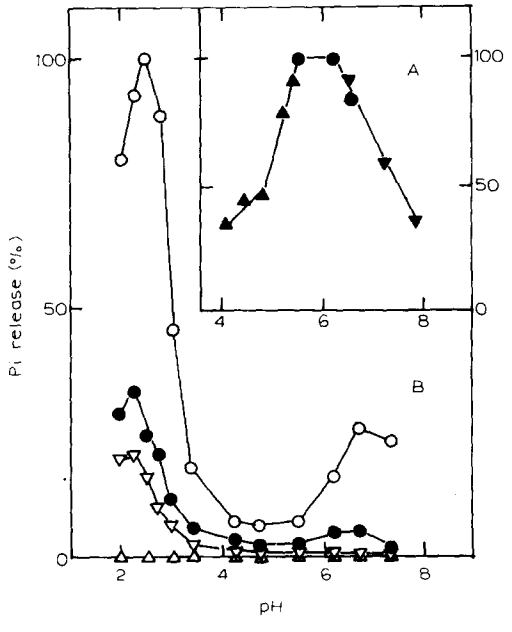


Fig. 1. Electron micrographs of thin sections of *Sulfolobus* whole cell (left) and its membrane P_0 (right). The membranes P_0 were prepared by repeated freeze-thaw procedures. Bar, 0.5 μm.



phosphates and other phosphoester compounds was examined. The results are summarized in Table II. Nucleoside triphosphates were hydrolyzed by all the cellular fractions. Compared with ATP, ADP was poorly hydrolyzed by the membrane fraction P_0. In the P_1 fraction, ADP did not inhibit ATP hydrolysis. Neither AMP, glucose 6-phosphate or fructose bisphosphate was hydrolyzed. The ATPase activity in P_1 was firmly associated with the cell membrane as described; this may be related to the energy transduction or active trans-

Fig. 2. pH dependence of the hydrolysis of PP_i, ATP, ADP and AMP, catalyzed by *Sulfolobus* membrane at 55°C. The reaction mixture contained in addition to the membranes: (A) 50 mM of either sodium acetate (▲), Tris-Pipes (●) or Tris-HCl (▼), 2 mM ATP and 2 mM $MgCl_2$. All reactions were carried out at 55°C. (B) 40 mM phthalate buffer, 2 mM substrate (○, PP_i; ●, ATP; ▽, ADP or △, AMP) and 2 mM $MgCl_2$. All reactions were carried out at 55°C for 20 min.

TABLE II

PHOSPHOHYDROLASE ACTIVITY WITH VARIOUS SUBSTRATES

Sulfolobus cell fractions, S_0, P_0, S_1 and P_1 were incubated with 2 mM of each substrate, 2 mM $MgCl_2$ and 50 mM Tris-HCl (pH 7) at 55°C for 20 min. The amount of P_i released was assayed. The relative activity was compared to that of ATP.

Substrate	Relative activity in the fractions (%)			
	S_0	P_0	S_1	P_1
ATP	100	100	100	100
GTP	85	103	152	135
ITP	122	108	106	75
CTP	–	–	–	35
UTP	–	–	–	54
ADP	48	12	17	8
AMP	–	0	0	0
ATP + ADP	–	–	–	115
Glucose 6-phosphate	–	–	–	0
Fructose bisphosphate	–	–	–	0
p-Nitrophenyl phosphate	275	2	3	1
PP_i	15000	240	1010	1100

port of the cell. Thus, in the following experiments, P_1 is referred to as the *Sulfolobus* membrane and characterized in detail.

Effects of temperature on Sulfolobus membrane ATPase

The *Sulfolobus* membrane ATPase was found quite thermostable. Even after the membrane was perincubated in 50 mM Tris-Pipes (pH 6.6) at 89°C for 20 min, the ATPase activity was fully retained. An Arrhenius plot was linear between 40 and 89°C, and the activation energy was calculated as 8.1 kcal/mol (data not shown). These results are in reasonable agreement with the thermophilic nature of *Sulfolobus*. Yang and Haug [22], however, reported that Arrhenius plots of *Thermoplasma* membrane ATPase revealed discontinuities in slope.

pH dependence of Sulfolobus membrane ATPase

Sulfolobus membrane ATPase activity was maximum at pH between 6 and 7 (Fig. 2A). In the presence of sulfate, this pH optimum shifted down to about 5 with concomitant activation of ATPase, as shown in Fig. 4. Similar results have been reported for *Thermoplasma* membrane ATPase [15].

However, the relationship between ATP hydrolyzing activity and various pH values (from 2 to 7.3), indicated the *Sulfolobus* membrane to have another pH optimum for ATP hydrolysis at about pH 2.5 (Fig. 2B). These pH optima were also found for inorganic pyrophosphatase activity (Fig. 2B). ADPase activity had only a single pH optimum around pH 2.5. AMP was not hydrolyzed at any pH. Although the ATP-hydrolyzing activity at pH 2.5 should be strictly that of apyrase, it is referred to as that of 'acid ATPase' in the following sections. Thus, the *Sulfolobus* membrane had both ATPase and inorganic pyrophosphatase, both of which occurring under acidic and neutral conditions.

Acid stability of Sulfolobus membrane ATPase and inorganic pyrophosphatase

The *Sulfolobus* membrane is a boundary between the acidic outside and neutral inside of the cell. Thus, the possibility as to whether its membrane bound ATPase and inorganic pyrophosphatase are acid stable was examined. The results are summarized in Table III. Following preincubation of the membrane in 0.1 M HCl at 20 or 55°C, both neutral ATPase and inorganic pyrophosphatase were irreversibly inactivated, whereas the acid ATPase and inorganic pyrophosphatase retained most of their activity.

Products of ATP hydrolysis

Fig. 3 shows the time-course of the hydrolysis products of ATP at pH 2.5 and 7. At pH 2.5, ATP was hydrolyzed via ADP to AMP. Good stoichiometry of $[P_i] = [ADP] + 2[AMP]$ was observed throughout the hydrolysis. At pH 7, however, ATP was hydrolyzed to ADP and P_i in equimolar stoichiometry. In contrast to *Thermoplasma* ATPase [15], only about 0.02 mM of AMP could be detected when 0.9 mM of ADP was produced. The linear time-course indicates that the product ADP was not inhibitory.

Effects of cations and anions on Sulfolobus membrane ATPase

Of the various divalent metal cations studied, magnesium was found the most effective and es-

TABLE III

ACID STABILITY OF *SULFOLOBUS* MEMBRANE ENZYMES

Sulfolobus membranes (5 mg protein/ml in 20 mM phthalate buffer, pH 7) were incubated with either 0.1 M HCl (final pH about 1) or 0.1 M NaCl (final pH 7) for 20 min at the temperatures indicated. Following the incubation, the reaction mixture was brought to pH 7 with 0.1 M NaOH, and an equal volume of buffer containing 100 mM Gly-HCl (pH 2.5) (or 100 mM Tris-HCl (pH 7.0) in the case of neutral enzymatic activity), 2 mM $MgCl_2$ and 2 mM of either PP_i or ATP was added immediately. After incubating this mixture at 70°C for 15 min, the amount of P_i was determined. The results are expressed as the percentage of each enzymatic activity of the membranes preincubated at pH 7, 20°C. PPase, inorganic pyrophosphatase.

Preincubation		Remaining activity (%)			
pH	Temp. (°C)	Acid PPase	Neutral PPase	Acid ATPase	Neutral ATPase
7	20	100	100	100	100
7	55	102	59	84	78
1	20	99	7	100	16
1	55	100	4	82	10

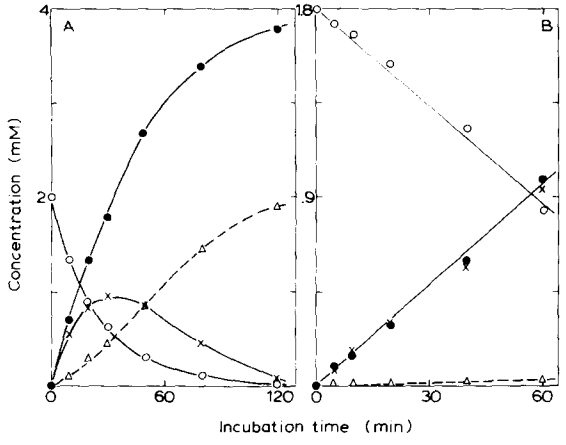


Fig. 3. Products of ATP hydrolysis catalyzed by *Sulfolobus* membrane at pH 2.5 (A) and pH 7 (B). Assay mixture contained in a final volume of 400 µl, 20 mM phthalate buffer (pH 2.5 or 7), 2 mM ATP, 1 mM $MgCl_2$ (or 1.8 mM ATP, 0.9 mM $MgCl_2$ in the case of the pH 7 experiment) and an appopriate amount of *Sulfolobus* membranes. The mixture was incubated at 55°C. At the time indicated, a 40 µl aliquot was withdrawn for P_i determination and nucleotide analysis on high performance liquid chromatography. Concentrations of ATP (○), ADP (×), AMP (△) and P_i (●) are indicated in mM. Control experiments, in which the membranes were omitted from the above assay mixture indicated 2 mM ATP to be hydrolyzed nonenzymatically to produce 0.2 mM P_i durin 120 min of incubation at 55°C, pH 2.5.

sential for neutral ATPase. Other metal cations could replace magnesium, but less effectively, as shown in Table IV. The optimal ratio of $MgCl_2$: ATP was 1 : 2 when the ATP concentration was between 0.5 and 4 mM (data not shown). Acid ATPase did not require divalent metal cat-

TABLE IV

EFFECT OF CATIONS AND ANIONS ON *SULFOLOBUS* MEMBRANE ACID AND NEUTRAL ATPases

Sulfolobus membranes were incubated at 55°C for 20 min with 2 mM ATP, either 50 mM Tris-HCl (pH 7.0) for neutral ATPase or 20 mM phthalate buffer (pH 2.5) for acid ATPase, and either divalent metal cations (A), 2 mM $MgCl_2$ and monovalent cations as indicated (B), or 2 mM $MgCl_2$ and 20 mM of the indicated anions (C). After incubation, P_i was estimated. The results are indicated as % of the value obtained when using 2 mM $MgCl_2$. All cations and anions were introduced as chloride and sodium salts, respectively. Acetate at pH 2.5 was adjusted by the addition of HCl.

	Relative activity (%)	
	Acid ATPase	Neutral ATPase
(A) Divalent cations (2 mM)		
Mg	100	100
Ca	97	40
Mn	53	87
Co	71	35
Ni	85	27
Cu	81	56
Zn	61	48
None	78	0
(B) Monovalent cations		
Na (40 mM)	105	101
(400 mM)	95	90
K (40 mM)	100	125
(400 mM)	104	62
Na (40 mM)+K (40 mM)	99	95
NH_4 (40 mM)	104	85
(400 mM)	67	60
(C) Anions (20 mM)		
HCO_3	–	110
HSO_3	29	190
SO_4	20	95
CH_3COO	100	100
HCOO	–	102
SCN	80	70
ClO_4	–	102
NO_3	87	106
F	3	6
Cl	105	117
Br	94	115

ions. Magnesium or calcium slightly activated the enzyme, while manganese and zinc were inhibitory. Monovalent cations had little effect on the ATPases.

Since certain anions such as carbonate, sulfite and sulfate affect F_1-ATPase of mitochondria [23,24] and bacteria [25], the effects of various anions on *Sulfolobus* membrane ATPase were examined. The results are summarised also in Table IV. Most of the anions had negligible effect on both the acid and the neutral ATPases. Fluoride inhibited both ATPases. Sulfate inhibited only the acid ATPase. Sulfite inhibited the acid ATPase and activated the neutral ATPase.

Sulfate and sulfite participate in the sulfur oxidizing pathway [26]. This prompted us to examine the effect of some sulfur compounds in detail. Sulfate strongly effected both ATPases as shown in Fig. 4. In the presence of sulfate, acid ATPase was inhibited without a change in its pH optimum. More than half of activity was inhibited by 2 mM sulfate. Neutral ATPase was apparently activated and its pH optimum shifted from 6.5 to 5 in the presence of sulfate. This activation was saturated at 10 mM sulfate. *Thermoplasma* membrane ATPase has been reported to be activated 'synergistically' with sulfate and magnesium [15]. The *Sulfolobus* membrane ATPase in the present study, however, was activated by sulfate alone, and was not effected by magnesium. The ATPases were also effected by sulfite but to a lesser degree. Thiocyanate and thiosulfate inhibited only acid ATPase. Elemental sulfur had only a little effect on the ATPases. Inorganic pyrophosphatase activity was inhibited by 20 mM sulfate by 20–30%, without change in their pH optima (data not shown).

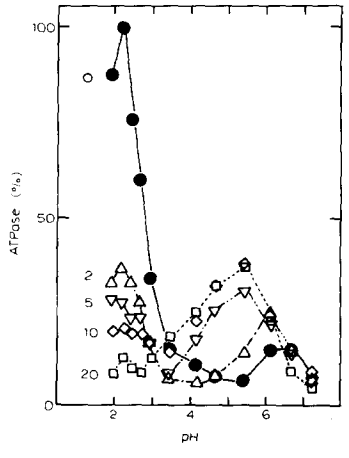


Fig. 4. Effect of sulfate on the relationship between pH and ATPase activity of the *Sulfolobus* membrane. ATPase activity was determined for the assay mixture containing 20 mM phthalate buffer, 2 mM ATP, 2 mM $MgCl_2$, the membranes, and the indicated concentration (in mM) of $MgSO_4$.

Effects of inhibitors

The specific inhibitors of mitochondrial and bacterial proton ATPase (F_0F_1), such as azide, ologomycin and DCCD [27], failed to inhibit *Sulfolobus* membrane ATPases (Table V). Ouabain, an inhibitor of $(Na^+ + K^+)$-ATPase [28,29] had no effect on *Sulfolobus* ATPase. Neither *N*-ethylmaleimide, *p*-chloromercuribenzoate (pCMB) or vanadate, inhibitors of intrinsic ATPases in various eukaryote membranes [30–33], had no significant inhibitory effect on the neutral or acid ATPase of *Sulfolobus* membrane (Table V).

Discussion

According to Harris [11], membrane-bound ATPase may be classified as intrinsic ATPase ($(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase) sensitive to *N*-ethylmaleimide, and extrinsic H^+-ATPase (F_1). In the present study, no typical characteristics of F_1 could be found in *Sulfolobus* membrane ATPases, judging from the lack of inhibition by ADP (Table II and Fig. 3), DCCD, azide and oligomycin (Table IV). The ATPases of *Sulfolobus* membrane were not extracted by the procedures used for the extraction of mesophilic F_1 [34], but their activity was released by such detergents as Triton X-100 and octaethyleneglycol monododecylether (Wakagi and Oshima, unpublished observation). The *Sulfolobus* membrane ATPases are apparently intrinsic, but differ from $(Na^+ + K^+)$-ATPase and Ca^{2+}-ATPase with respect to cation requirements (Table IV) and inhibitor sensitivity (Table V).

Thus, *Sulfolobus* membrane ATPases do not fit above classifications. Neutral ATPase was remarkably stimulated by sulfate, with a shift of pH optimum (Fig. 4). Similar ATPase has been reported in *Thermoplasma*, but acidic ATPase in

TABLE V

EFFECTS OF INHIBITORS ON *SULFOLOBUS* MEMBRANE ATPase

Sulfolobus membranes (1 mg protein/ml) in 5 mM Tris-HCl (pH 7.5) were incubated with each inhibitor at 37°C for 30 min, and then 1/100 volume of 0.2 M ATP-Mg and 1/10 volume of either 0.5 M Gly-HCl (pH 2.5) or 0.5 M Tris-HCl (pH 7.0) was added. The mixture was incubated at 55°C for 20 min. The released P_i was indicated as the % of the value obtained in the absence of inhibitor.

Inhibitor		Relative activity (%)	
		acid ATPase	neutral ATPase
None		100	100
NaN_3	(10 mM)	97	86
Oligomycin	(0.1 mM)	96	86
DCCD	(1 mM)	94	63
Ouabain	(0.1 mM)	89	97
N-Ethylmaleimide			
	(1 mM)	93	73
pCMB	(0.1 mM)	101	104
Na_3VO_4	(0.1 mM)	90	64
NaF	(2 mM)	82	86
	(20 mM)	6	6

this organism is unknown [15]. The effect of sulfate on the membrane ATPase of these thermoacidophiles may be related to certain aspects of sulfur metabolism, since, according to Searcy and Whatley [15], *Thermoplasma* ATPase may be a sulfate-exporting translocase. The membrane bound ATPase of *Thiobacillus thiooxidans* has been reported to be activated by sulfite with a shift of its optimal pH [35]. Mitochondrial F_1 is also activated by sulfite [23], but the membrane ATPase of thiobacilli apparently differs from F_1 [36].

A different branch of archaebacteria among methanogens, halophiles and thermoacidophiles has been proposed on the basis of a study of their 16S rRNA [3,37], ferredoxin [38], DNA-dependent RNA polymerase [39,40] and ribosome [10]. Thus, it is reasonable that different ATPases occur among archaebacteria. The similarity of membrane bound ATPase in *Sulfolobus* and *Thermoplasma* is consistent with the above proposal.

It is surprising that the *Sulfolobus* membrane has an acid ATPase with an optimum pH about 2.5 (Fig. 2), which is close to the optimum pH for growth. The high stability of this enzyme toward acid makes it possible for it to be present on the outer surface of the membrane and consequently may be an ectoenzyme [41]. The acid lability of neutral ATPase (Table III) suggests it to be present on the inner surface of the membrane. Since the cytoplasmic enzymes of *Sulfolobus* so far examined were inactive below pH 6, the intracellular pH should be nearly neutral, as in the case of other acidophiles [42–44].

The inorganic pyrophosphatase activity associated with *Sulfolobus* membrane also showed two pH optima (Fig. 2) and acid stability (Table III) similar to those of ATPase activity. That inorganic pyrophosphatase and ATPase derive from the same enzyme still remains a possibility. The membrane-bound inorganic pyrophosphatase of photosynthetic bacteria [45,46] and mitochondria [47] has been reported to couple with oxidative phosphorylation.

For an understanding of the physiological roles of ATPases and inorganic pyrophosphatases in the *Sulfolobus* membrane, additional research will be necessary. The purification of these enzymes is now being carried out at our laboratory.

References

1 Brock, T.D., Brock, K.M., Belly, R.T. and Weiss, R.L. (1972) Arch. Microbiol. 84, 55–68
2 Shivvers, D.W. and Brock, T.D. (1973) J. Bacteriol. 114, 706–710
3 Fox, G.E., Stackenbrandt, E., Hespell, R.B., Gibson, J., Maniloff, J., Dyer, T.A., Wolfe, R.S., Blach, W.E., Tanner, R.S., Magrum, L.J., Zablen, L.B., Stahl, D.A., Luehrsen, K.R., Chen, K.N. and Woese, C.R. (1980) Science 209, 457–463
4 Langworthy, T.A., Mayberry, W.R. and Smith, P.F. (1974) J. Bacteriol. 119, 275–284
5 Langworthy, T.A. (1977) J. Bacteriol. 130, 1326–1332
6 Woese, C. and Fox, G.E. (1977) Proc. Natl. Acad. Sci. USA 74, 5088–5090
7 Searcy, D.G., Stein, D.B. and Green, G.R. (1978) BioSystems 10, 19–28
8 Hori, H. and Osawa, S. (1979) Proc. Natl. Acad. Sci. USA 76, 380–388
9 Van Alen, L.M. and Maiorana, V.C. (1980) Nature 287, 248–250
10 Lake, J.A., Henderson, E., Oakes, M. and Clark, M. (1984) Proc. Natl. Acad. Sci. USA 81, 3786–3790
11 Harris, D.A. (1981) BioSystems 14, 113–121
12 Spiott, G.D. and Jarrell, K.F. (1982) Can. J. Microbiol. 28, 982–986
13 Doddema, H.J., Hutten, T.J., Van der Drift, C. and Vogels, G.D. (1978) J. Bacteriol. 136, 19–23

14 Doddema, H.J. (1979) J. Bacteriol. 140, 1081–1089
15 Searcy, D.G. and Whatley, F.R. (1982) Zbl. Bakt. Hyg., I. Abt. Orig. C3, 245–257
16 Inatomi, K., Ohba, M. and Oshima, T. (1983) Chem. Lett., 1191–1194
17 Lin, T.I. and Morales, M.F. (1977) Anal. Biochem. 77, 10–17
18 Rathbun, W.B. and Betlach, M.V. (1969) Anal. Biochem. 28, 436–445
19 Klingenberg, M. (1963) in Methods of Enzymatic Analysis, 1st Edn. (Bergmeyer, H.U., ed.), pp. 528–530, Academic Press, New York
20 Linhardt, K. and Walter, K. (1963) in Methods of Enzymatic Analysis, 1st Edn. (Bergmeyer, H.U., ed.), pp. 779–787, Academic Press, New York.
21 Wang, C.H. and Smith, R.L. (1975) Anal. Biochem. 63, 414–417
22 Yang, L. and Haug, A. (1979) Biochim. Biophys. Acta 573, 308–320
23 Ebel, R.E. and Lardy, H.A. (1975) J. Biol. Chem. 250, 191–195
24 Wakagi, T. and Ohta, T. (1981) J. Biochem. (Tokyo) 89, 1205–1213
25 Webster, G.D. and Jackson, G.D. (1978) Biochim. Biophys. Acta 503, 135–154
26 Roy, A.B. and Trudinger, P.A. (1970) The Biochemistry of Inorganic Compounds of Sulfur, Cambridge University Press, London
27 Hoppe, J. and Sebald, W. (1981) in Chemiosmotic Proton Circuits in Biological Membranes (Skulachev, V.P. and Hide, P.C., eds.), pp. 449–458, Addison-Wesley, Reading, MA
28 Schwaltz, A., Wintmer, K., Grupp, G., Grupp, I., Adams, R., and Lee, S.W. (1982) Ann. N.Y. Acad. Sci. 402, 253–271
29 Cantley, L., Carilli, C.T., Farley, R.A. and Perlman, D.M. (1982) Ann. N.Y. Acad. Sci. 402, 289–291
30 O'Neal, S.G., Rhoads, D.B. and Racker, E. (1979) Biochem. Biophys. Res. Commun. 89, 845–850
31 Scarborough, G.A. (1977) Arch. Biochem. Biophys. 180, 384–393
32 Brooker, R.J. and Slayman, C.W. (1982) J. Biol. Chem. 257, 12051–12055
33 De Pont, J.J.H.H.M. and Bonting, S.L. (1981) in Membrane Transport (Bonting, S.L. and De Pont, J.J.H.H.M., eds.), pp. 209–234, Elsevier/North-Holland Biomedical Press, Amsterdam
34 Linnet, P.E., Mitchell, A.D., Partis, M.D. and Beechey, R.B. (1979) Methods Enzymol. 55, 337–343
35 Marunouchi, T. and Mori, T. (1967) J. Biochem. (Tokyo) 62, 401–407
36 Ingledew, W.J. (1982) Biochim. Biophys. Acta 683, 89–117
37 Tu, J., Prangishvilli, D., Huber, H., Wildgruber, G., Zillig, W. and Stetter, K.O. (1982) J. Mol. Evol. 18, 109–114
38 Kerscher, L., Nowitzki, S. and Oesterhelt, D. (1982) Eur. J. Biochem. 128, 223–230
39 Zillig, W., Stetter, K.O. and Janekovic, D. (1979) Eur. J. Biochem. 96, 597–604
40 Zillig, W., Stetter, K.O., Wunderl, S., Schulz, W., Priess, H. and Scholz, I. (1980) Arch. Microbiol. 125, 259–269
41 Stanley, K.K., Newby, A.C. and Luzio, J.P. (1982) Trends Biochem. Sci. 7, 145–147
42 Padan, E., Zilberstein, D. and Schuldinger, S. (1981) Biochim. Biophys. Acta 650, 151–166
43 Oshima, T., Arakawa, H. and Baba, M. (1977) J. Biochem. (Tokyo) 81, 1107–1113
44 Searcy, D.G. (1976) Biochim. Biophys. Acta 451, 278–286
45 Reeves, R.E. (1976) Trends Biochem. Sci. 1, 43–55
46 Lahti, R. (1983) Microbiol. Rev. 47, 169–179
47 Mansurova, S.E., Shakhov, Y.A. and Kulaev, I.S. (1977) FEBS Lett. 74, 31–34