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PROPERTIES AND FUNCTION OF CLOSTRIDIAL MEMBRANE ATPase

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SUMMARY

ATPase (ATP phosphohydrolase, EC 3.6.1.3) was detected in the membrane fraction of the strict anaerobic bacterium, *Clostridium pasteurianum*. About 70 % of the total activity was found in the particulate fraction. The enzyme was Mg^{2+} dependent; Co^{2+} and Mn^{2+} but not Ca^{2+} could replace Mg^{2+} to some extent; the activation by Mg^{2+} was slightly antagonized by Ca^{2+}. Even in the presence of Mg^{2+}, Na^+ or K^+ had no stimulatory effect. The ATPase reaction was effectively inhibited by one of its products, ADP, and only slightly by the other product, inorganic phosphate. Of the nucleoside triphosphates tested ATP was hydrolyzed with highest affinity ($[S]_{0.5\,V} = 1.3$ mM) and maximal activity (120 U/g). The ATPase activity could be nearly completely solubilized by treatment of the membranes with 2 M LiCl in the absence of Mg^{2+}. Solubilization, however, led to instability of the enzyme.

The clostridial solubilized and membrane-bound ATPase showed different properties similar to the "allotopic" properties of mitochondrial and other bacterial ATPases. The membrane-bound ATPase in contrast to the soluble ATPase was sensitive to the ATPase inhibitor dicyclohexylcarbodiimide (DCCD). DCCD, at 10^{-4} M, led to 80 % inhibition of the membrane-bound enzyme; oligomycin, ouabain, or NaN_3 had no effect. The membrane-bound ATPase could not be stimulated by trypsin pretreatment.

Since none of the mono- or divalent cations had any truly stimulatory effect, and since a pH gradient (interior alkaline), which was sensitive to the ATPase inhibitor DCCD, was maintained during growth of *C. pasteurianum*, it was concluded that the function of the clostridial ATPase was the same as that of the rather similar mitochondrial enzyme, namely H^+ translocation. A H^+-translocating, ATP-consuming ATPase appears to be intrinsic equipment of all prokaryotic cells and as such to be phylogenetically very old; in the course of evolution the enzyme might have been developed to a H^+-(re)translocating, ATP-forming ATPase as probably realized in aerobic bacteria, mitochondria and chloroplasts.

INTRODUCTION

It was shown recently that *C. pasteurianum* generates and maintains, during growth, a pH gradient (interior alkaline), which was abolished by the proton con-

ductor carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and the ATPase inhibitor *N,N'*-dicyclohexylcarbodiimide (DCCD). The pH gradient could not be found in cells depleted of an energy source [1]. These results suggested that the pH gradient was formed by an ATPase-driven extrusion of protons from the cells.

Since with prokaryonts ATPase activity has so far been demonstrated only in aerobic species [2–19], in which they might be involved in electron transport phosphorylation, and in atypical anaerobes [20–24], such as the reverted aerobe [25] *Streptococcus faecalis*, in which it might support ion and substrate transport [26, 27], it appeared necessary to check whether ATPase activity is present also in the strict anaerobe *C. pasteurianum* and whether its function might be the generation of the pH gradient observed. In addition, the question was to be clarified whether a H^+-translocating ATPase has been retained in *S. faecalis* as a relict during evolutionary reversion from aerobiosis to anaerobiosis, or whether it is in general an intrinsic enzyme of all prokaryonts, thus already occurring in the strictly anaerobic *Clostridia*, which resemble best in their biological properties (e.g. type of energy metabolism) the earliest forms of microorganisms in evolution [28].

In this paper, evidence is presented for a Mg^{2+}-dependent, membrane-associated ATPase in *C. pasteurianum*. The enzyme is not influenced by Na^+, K^+ or Ca^{2+}, it appears to function as an electrogenic H^+ translocator. These findings suggest that a H^+-translocating ATPase is an intrinsic enzyme of all prokaryonts. Its probable physiological role in anaerobic bacteria is the generation of a transmembrane pH gradient (interior alkaline) and of an electrical potential difference (interior negative), both required for various types of transport processes.

MATERIALS AND METHODS

1. *Chemicals*

Reagent grade chemicals were used throughout. Enzymes, substrates and chemicals were purchased from Boehringer Mannheim, and from E. Merck AG, Darmstadt.

2. *Culture of Clostridia*

C. pasteurianum (ATCC 6013) was grown in 1-l flasks on synthetic glucose/salt minimal medium as described by Lovenberg et al. [29]. The bacteria were harvested at the end of the growth phase by centrifugation at 6 000 × *g* for 15 min at 0 °C. The yield averaged 5 g of wet cells per l of culture. The bacteria were washed once with 20 ml of ice-cold distilled water per g wet wt. of cells by centrifugation at 6 000 × *g* for 15 min at 0 °C.

3. *Preparation of membranes*

1 g wet wt. of bacteria was suspended in 4 ml incubation buffer pH 7.5 containing 0.1 M Tris · HCl/0.5 M sucrose/20 mM $MgCl_2$/0.2 mg of lysozyme per ml. The suspension was incubated for 45 min at +37 °C with occasional stirring by a magnetic paddle. After addition of 0.1 mg of lysozyme per ml the suspension was kept for another 45 min at +37 °C to obtain complete protoplast formation. The protoplasts were collected by centrifugation at 16 000 × *g* for 20 min at 0 °C [22]. The pellet was suspended to the original volume with hypotonic buffer pH

7.5 containing 1 mM Tris · HCl and 1 mM $MgCl_2$ and incubated at +37 °C for 10 min after addition of 30 μg of DNAase per ml of suspension. The protoplasts were completely lysed by osmotic shock as controlled by phase-contrast microscopy. The lysate, which contained the total ATPase activity, was then centrifuged at 130 000 × *g* for 60 min at 0 °C. The pellet was suspended to the original volume with buffer pH 7.5 containing 1 mM Tris · HCl and 1 mM $MgCl_2$. This suspension is referred to as crude membrane ATPase, which was only used in experiments concerning enzyme activity distribution. All further experiments were carried out with washed membranes. For this purpose the crude membranes were submitted to 100 000 × *g* centrifugation for 30 min at 0 °C and resuspended in 1 mM Tris · HCl pH 7.5 with 1 mM $MgCl_2$. This suspension is referred to as membrane ATPase. The protein content of the fractions was determined by the method of Lowry et al. [30].

4. *Solubilization of membrane ATPase*

(*i*) *Treatment of membranes with buffer of high ionic strength.* Membrane ATPase was centrifuged at 100 000 × *g* for 30 min at 0 °C and resuspended with Mg^{2+}-free buffer pH 7.5 containing 250 mM Tris · HCl and 2 M LiCl. The membranes were stirred at room temperature for 30 min. After 100 000 × *g* centrifugation for 30 min at 0 °C nearly the complete ATPase activity appeared in the supernatant, while the membrane fraction was practically inactive. The activity in the 100 000 × *g* supernatant is referred to as soluble ATPase. For DCCD inhibition experiments this fraction had to be desalted by Sephadex G 25 column passage (Whatman column, length 10 cm, diameter 1 cm) in order to exclude inhibitory effects of high ionic strengths.

Furthermore different methods for solubilization were applied:

(*ii*) "*Shock-wash*" *procedure with* Mg^{2+}*-free buffer.* Membrane ATPase was washed 15 times with 1 mM Tris · HCl pH 7.5 by repeated centrifugation at 100 000 × *g* for 30 min at 0 °C. The resulting supernatants and pellets were assayed for ATPase activity.

(*iii*) "*Shock-wash*" *procedure with EDTA buffer.* The procedure was the same as in (ii) except that membrane ATPase was washed 5 times with 1 mM Tris · HCl pH 7.5 containing 1 mM EDTA.

(*iv*) *Sonication.* Membrane ATPase was sonified at 0 °C for 10 min at 20 000 kHz with a Branson sonifier J 17 A. Sonication was carried out in periods of 30 s with 30-s intervals to prevent thermal damage of the preparation. The sonicated suspension was then centrifuged and assayed as described for procedure i.

(*v*) *Detergents.* After centrifugation at 100 000 × *g* for 30 min at 0 °C the membrane ATPase was suspended to the original volume in 1 mM Tris · HCl pH 7.5 containing alternatively 0.05 % or 1 % sodium deoxycholate, 0.1 % sodium dodecyl sulfate, or 10 % Triton X-100 (v/v), respectively. The suspensions were incubated for 30 min at room temperature and then centrifuged at 100 000 × *g* for 30 min. The resulting supernatants and pellets, the latter being resuspended with 1 mM Tris · HCl pH 7.5, were assayed for ATPase activity.

5. *ATPase assay*

Both ADP and inorganic phosphate, the products of the ATPase reaction, were determined in parallel in a test system containing 100 mM Tris · HCl pH 7.5;

5 mM ATP, disodium salt; 5 mM $MgCl_2$; ATP-regenerating system consisting of 2 mM phosphoenolpyruvate, tricyclohexylammonium salt; 0.5 mM NADH, disodium salt; 20 units of pyruvate kinase; 30 units of lactate dehydrogenase; water to 1.0 ml. In experiment with ADP as an inhibitor or substrate of the ATPase reaction the assay was devoid of an ATP-regenerating system. The reaction was started with protein and run at +37 °C. Formation of ADP was monitored photometrically at 366 nm. Inorganic phosphate was determined in a parallel assay. The reaction was run for 5 min at +37 °C and stopped by addition of 100 µl 50 % trichloroacetic acid. After removal of the denatured protein by centrifugation inorganic phosphate (P_i) in the supernatant was determined by the method of Fiske and SubbaRow [31]. The values obtained were corrected against P_i liberated from the nucleotides and assay components under the acidic conditions of the Fiske-SubbaRow procedure. One unit of activity is defined as the amount of enzyme which catalyzes the formation of 1 µmol of ADP or P_i per min under the conditions described above. The ATPase assay was not interfered by adenylate kinase, since (i) the preparations of membrane ATPase were practically free of adenlyate kinase and (ii) pyruvate kinase added in the test was so much higher than the negligable residual adenylate kinase activity of the preparations that the latter could not compete for the ADP formed by the ATPase reaction. The results obtained by the ADP- and P_i-determination were identical. For the investigation of alkali cation effects both sodium-NADH and sodium-ATP had to be passed through a column containing 3 g of Dowex 50W X 8, mesh 100–200, ammonium form, in order to obtain the corresponding ammonium salts.

A possible masking of ATPase was studied by incubating the enzyme preparation in the presence of trypsin (100 µg/ml assay) for 10 min at +37 °C and at pH 7.5 before starting the test reaction.

6. *Inhibition of ATPase*

Inhibition experiments were carried out with DCCD, oligomycin, and ouabain in a concentration range of 10^{-8}–10^{-3} M each, and with NaN_3 up to 10^{-2} M by incubation of the enzyme for 15 min at +37 °C before starting the reaction. Since DCCD and oligomycin were dissolved in pure ethanol and the ethanol concentration in the preincubation assay amounted to 1 %, a simultaneous preincubation with ethanol alone was carried out to exclude any solvent effect.

RESULTS

1. *Activity distribution in soluble and particulate fractions*

For cellular localization of ATPase the total activity of the osmotic shock lysate was compared with both the activities found in the supernatant and in the pellet after 130 000 × *g* ultracentrifugation. The data of four typical localization experiments suggest that the amount of apparent ATPase activity in the membrane fraction averages 70 % (Table I). In contrast to the stable membrane-bound activity, the ATPase of the crude soluble fraction proved to be unstable. Since the ADP and P_i assays yielded different results with the crude soluble ATPase, this activity rather reflects an apparent than a genuine ATPase. Due to the identical results obtained by

TABLE I

ACTIVITY DISTRIBUTION IN THE SOLUBLE AND PARTICULATE FRACTIONS OF *C. PASTEURIANUM*

The enzyme was assayed with the phosphate determination in the presence of 5 mM Mg^{2+} · ATP. Data are obtained from four typical experiments.

Cell fraction	Enzyme activity (μmol $P_i \cdot min^{-1} \cdot ml^{-1}$)			
Osmotic shock lysate (total activity)	2.6	1.6	2.3	1.7
Crude 130 000 × *g* supernatant	0.23	0.23	0.6	0.23
Particulate fraction	2.4	0.92	1.6	0.84
% of total activity	92 %	58 %	70 %	49 %

ADP and P_i determination with the particulate fraction it is very likely that the genuine ATPase of *C. pasteurianum* is essentially membrane bound.

2. *Solubilization of membrane ATPase*

Various attempts to solubilize the membrane ATPase were made: (i) by treatment of the membranes with Mg^{2+}-free buffer of high ionic strength, (ii, iii) by "shock-wash" procedures with Mg^{2+}-free buffers of low ionic strength with and without EDTA, (iv) by sonication, and (v) by detergents.

The ATPase was nearly completely released from the membrane by one washing step with 250 mM Tris · HCl pH 7.5 containing 2 M LiCl. The solubilized enzyme was not stable, similar to that of the crude soluble fraction. The instability was also observed after desalting the solubilized enzyme preparation by Sephadex G 25 column passage, so that a destabilizing effect of high ionic strength could be excluded. The activity of the solubilized enzyme decreased to 10 % of the initial value within 3 h at 0 °C. An even more rapid inactivation took place at +25 °C. The instability could not be prevented by addition of 20 mM $MgCl_2$ or 1 mg bovine serum albumin per ml. Rebinding experiments under various conditions with the labile solubilized enzyme were so far not successful.

Repeated washing of the membranes with 1 mM Tris · HCl pH 7.5 led to solubilization of about 5 % of the membrane ATPase activity per washing step as also observed with *Staphylococcus aureus* [8]. The bulk of the enzyme could not be released at one distinct step of the "shock-wash" procedure as observed with *S. faecalis* [20–22], *E. coli* [14], *Bacillus megaterium* [5] and *Proteus mirabilis* [16]. After the 13th wash the clostridial membranes were depleted of ATPase activity.

No solubilization of ATPase occurred by treating the membranes with sonication or detergents such as deoxycholate, Triton X-100, and sodium dodecyl sulfate. Moreover, the activities of both the soluble and particulate fraction were completely destroyed after treatment with sonication or Triton X-100. A possible inhibitory effect of the detergents on the ATPase assay was excluded in a separate experiment.

3. *Half saturation concentration and maximal activity*

The reaction catalyzed by membrane ATPase was linear with time up to 20 min and linear with protein up to 0.5 mg per ml. The enzyme exhibited a Michaelis-

TABLE II

SUBSTRATE SPECIFICITY OF CLOSTRIDIAL MEMBRANE ATPase

The enzyme was assayed with the phosphate determination. The relative activity was tested with the nucleotides at 5.0 mM with a nucleotide/Mg^{2+} ratio of 1. With ADP as the substrate the ATP-regenerating system of the assay was omitted.

Substrate	Substrate affinity $[S]_{0.5\,v}$ (mM)	Relative activity (%)
ATP	1.3	100
ADP	—	0.8
AMP	—	—
GTP	1.6	50
ITP	—	9
UTP	2.6	68
CTP	1.5	38

Menten-type hyperbolic dependence on substrate concentration. The $[S]_{0.5\,v}$ value for ATP was 1.3 mM at a molar ATP/Mg^{2+} ratio of 1 (Table II). Maximal enzyme activity was obtained at ATP concentrations of 5 mM. The specific activity averaged 120 U per g of membrane protein.

4. *Substrate specificity*

Maximal activity and highest affinity was observed with ATP as the substrate. Various other nucleotides were split, too (Table II). No unspecific phosphatase activity was detectable in the enzyme preparations as tested with *p*-nitrophenyl-phosphate as the substrate in the concentration range up to 2 mM.

5. *Cation requirement*

The clostridial membrane ATPase required Mg^{2+} for activity at pH 7.5 and 9.0; the reaction velocity reached a maximum at an ATP/Mg^{2+} ratio of 1. These findings make it appear likely that Mg^{2+} should be necessary for the formation of the active $Mg^{2+} \cdot$ ATP substrate complex rather than for direct enzyme stimulation. Mn^{2+} and Co^{2+} but not Ca^{2+} could replace Mg^{2+} to a lesser extent. The action of Mg^{2+} could even be slightly antagonized by increasing concentrations of Ca^{2+} (Table III). Lack of activity with Ca^{2+} and inhibition by Ca^{2+} would indicate that the clostridial ATPase belongs to the type II metal enzymes, which bind the metal-substrate complex via a metal bridge rather than via a substrate bridge [32, 33]. Na^{+} and K^{+}, in combination or alone, had neither a stimulating nor an inhibiting effect on the enzyme (Table III).

6. *pH-dependence*

The enzyme showed a broad pH optimum with near maximal activities in the range between 7.0 and 8.5.

7. *Product inhibition by ADP and inorganic phosphate*

ADP, one of the products of the ATPase reaction, had an inhibitory effect on enzyme action. It was concluded from a Lineweaver-Burk plot that ADP was an

TABLE III

EFFECT OF CATIONS ON THE CLOSTRIDIAL MEMBRANE ATPase

The enzyme activity obtained with ATP and Mg^{2+}, 2 mM each, was taken as 100 %. The effect of the mono- and divalent cations was tested with both the ADP and P_i assay, and with ATP and NADH as the ammonium salts instead of the corresponding sodium salts. The presence of NH_4^+ assured a non-limiting activity of the indicator enzyme pyruvate kinase.

Added cations	Relative activity (%)
None	0
Divalent cations	
Mg^{2+} 2 mM	100
Co^{2+} 2 mM	33
Mn^{2+} 2 mM	70
Ca^{2+} 2 mM	0
Ca^{2+} 2 mM, Mg^{2+} 2 mM	69
Monovalent cations	
Mg^{2+} 2 mM, Na^+ 100 mM	100
Mg^{2+} 2 mM, K^+ 100 mM	100
Mg^{2+} 2 mM, Na^+ 100 mM, K^+ 5 mM	100
Mg^{2+} 2 mM, Na^+ 100 mM, K^+ 100 mM	100

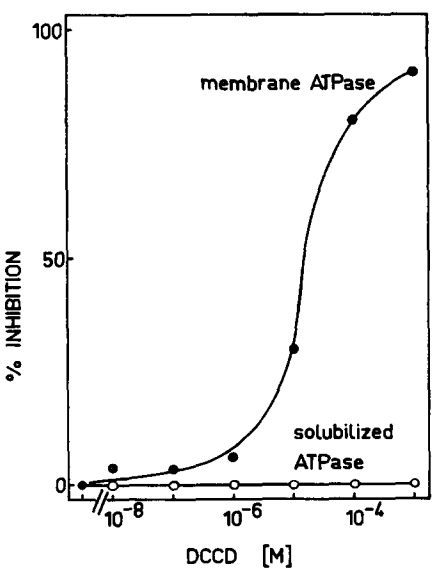

Fig. 1. DCCD inhibition of clostridial ATPase. ATPase was solubilized by treatment of the membranes with buffer containing 250 mM Tris · HCl pH 7.5, and 2 M LiCl. The desalted (Sephadex G 25 column passage) solubilized ATPase preparation and membrane ATPase were incubated for 15 min at +37 °C in the presence of DCCD before starting the reaction. The value of enzyme activity in the absence of DCCD was taken as 100 %. Photometrical ADP determination: start with 0.4 mg of solubilized or membrane ATPase, respectively. (○—○) solubilized ATPase, (●—●) membrane ATPase. Ethanol as the solvent for DCCD had no effect on the enzyme activity.

effective inhibitor probably replacing ATP as the substrate from its binding site. Inorganic phosphate, the other product, also exerted a slight inhibitory effect. In the presence of 50 mM inorganic phosphate the maximal enzyme activity decreased to 80 % of its initial value. A change in enzyme affinity was not observed.

8. *Lack of trypsin demasking of membrane ATPase*

A stimulatory effect of trypsin on membrane ATPase, attributed to a demasking of additional activity from the membrane, was observed with *Escherichia coli* [15] and *Micrococcus lysodeikticus* [3]. However, the clostridial membrane ATPase activity decreased from 100 % to 85 % during a 10 min incubation with trypsin; the solubilized activity was completely destroyed under this condition. Thus, demasking of membrane ATPase by partial proteolysis of a hypothetical ATPase inhibitor could not be detected with *C. pasteurianum.*

9. *DCCD inhibition*

Pretreatment with the ATPase inhibitor DCCD at 10^{-4} M led to 80 % inhibition of the membrane ATPase (Fig. 1). In contrast, the solubilized ATPase, which could be removed from the membrane with buffer of high ionic strength, was not sensitive to DCCD. This result is in line with the earlier conclusion drawn from work with mitochondria [34, 35] and bacteria [36, 37] that inhibition by DCCD is caused by covalent binding to a membrane constituent of the ATPase assembly rather than by direct action on the ATPase protein itself. The different behaviour of the clostridial solubilized and membrane-bound ATPase towards DCCD resembles the "allotopic" properties of mitochondrial [38, 39] and other bacterial ATPases [4, 10–12, 16, 37]. Preincubation of solubilized and membrane ATPase for 15 min with oligomycin and ouabain in a concentration range of 10^{-8}–10^{-3} M each, and with NaN_3 in a concentration range of 10^{-8}–10^{-2} M had no inhibitory effect on either activity.

DISCUSSION

Properties

It was shown in this investigation that cell-free extracts of *C. pasteurianum* contain ATPase activity. About 70 % of the total ATPase activity was found in the membrane fraction after gentle osmotic lysis of protoplasts. This value is in agreement with data obtained from *S. faecalis* [20], *Vibrio parahaemolyticus* [18] and *M. lysodeikticus* [3]. The activity of the soluble fraction was unstable and due rather to an apparent than a genuine ATPase, since different results were obtained by the ADP and P_i determination. In contrast, identical values were obtained with the particulate fraction. These findings strongly suggest that only the membrane-bound activity is a genuine ATPase.

There appears to be a considerable variety of divalent cation effects on bacterial ATPases. Around pH 7 the ATPase of *C. pasteurianum* required Mg^{2+} for the formation of the $Mg^{2+} \cdot$ ATP substrate complex; Mg^{2+} could not be replaced by Ca^{2+}. A similar behaviour was reported for the ATPases from *S. faecalis* [20], *Streptococcus pyogenes* [23], *V. parahaemolyticus* [18] and a *Pseudomonas* species [17]. In contrast, the ATPases from *M. lysodeikticus* [2], *B. megaterium* [4], *Bacillus subtilis* [6] and *Proteus mirabilis* [16] as well as from mitochondria [38] were found to be

operative also, sometimes even more effectively, with Ca^{2+}. With *E. coli* [9, 11, 14] the divalent cation requirement was shown to be markedly pH dependent: at pH 7 the enzyme functioned exclusively with Mg^{2+}; yet at pH 9 it worked also without Mg^{2+} or Ca^{2+} and could slightly but equally well be stimulated by Mg^{2+} and Ca^{2+}. With the clostridial ATPase there was no divalent cation independent activity nor could Ca^{2+} substitute for Mg^{2+} even at the more alkaline pH of 9. However, the physiological significance of cation effects at pH 9 remains open, since in vivo the intracellular pH does not appear to be so alkaline [1, 40].

Also with respect to the effect of Na^+ and K^+ on bacterial ATPases, remarkable differences appear to exist. The clostridial ATPase was not affected by single or combined application of Na^+ or K^+ nor was it inhibited by ouabain, a typical inhibitor of the mammalian $(Na^+ + K^+)$-translocating ATPase. This finding is in agreement with the results obtained with the ATPases from *Lactobacillus arabinosus* [24], *B. megaterium* [4], *B. stearothermophilus* [7], a *Pseudomonas* species [17] and *Thiobacillus thiooxidans* [19]. In contrast, the enzymes from *S. faecalis* [20], *Proteus mirabilis* [16], *B. subtilis* [6], *Staphylococcus aureus* [8] and *V. parahaemolyticus* [18] could be stimulated to varying extents at alkali metal concentrations in the range from 50 mM to more than 100 mM. However, ouabain had no effect on the ATPases in these organisms either. Finally, conflicting results have been reported for the ATPase from *E. coli*. On one hand inhibition by Na^+ alone [11] and by Na^+ and K^+ alone and together [14] has been noted; on the other hand a very small ouabain-sensitive activation by Na^+ plus K^+ ($< 5\%$) has been observed [13]. The physiological significance of a possible $(Na^+ + K^+)$-translocating ATPase in *E. coli*, indicated by the latter finding, remains questionable, since the K^+ translocation in this organism [11] and other bacteria [26, 41] is known to be independent of the presence and transport of Na^+.

In conclusion it is proposed, that the variability of the effects of Mg^{2+} and Ca^{2+} as well as of Na^+ and K^+ on bacterial ATPases probably reflects different catalytic mechanisms, i.e. enzyme-substrate-metal, enzyme-metal-substrate or metal-enzyme-substrate complex formation [32, 33] rather than different functions, i.e. H^+-, Na^+/K^+- or Mg^{2+}/Ca^{2+}-transport.

Function

The absence of any truly stimulatory effect of the mono- or divalent cations tested and the inhibition by DCCD suggest that the clostridial membrane ATPase serves as H^+-translocating enzyme required to maintain the DCCD-sensitive pH gradient observed [1]. Since growth was inhibited by low concentrations of DCCD [1], it appears likely that the ATPase is an essential enzyme for the growing bacterium. Its function in general would be to provide a link between metabolism and transport via the electrogenic extrusion of protons from the cell, i.e. via the formation and maintenance of a pH-gradient (interior alkaline) and concomitantly of an electrical potential difference (interior negative) [41]. Both would be required for various types of transport processes (Fig. 2).

Since strict anaerobes, such as *C. pasteurianum*, should best resemble the earliest forms of microorganisms, it may be concluded that a H^+-translocating ATPase is an intrinsic outfit of all prokaryotic cells rather than an accidental retainment from oxidative phosphorylation, a possibility that could not be excluded in the case

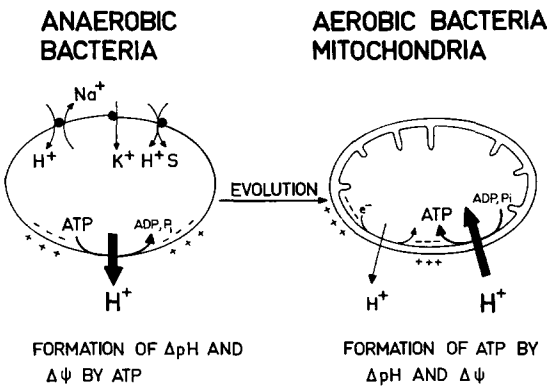

Fig. 2. Hypothetical change of ATPase function during evolution. ΔpH = pH gradient (interior alkaline); $\Delta\psi$ = electrical potential difference (interior negative); S = neutral substrate; $e^- \rightarrow$ = electron transport in respiratory chain generating ΔpH and $\Delta\psi$.

of the revertant *S. faecalis*.

Due to the close relationship of mitochondrial and bacterial ATPases, now also demonstrated with the phylogenetically very old, strict anaerobe *C. pasteurianum*, it is tempting to speculate that the H^+-ATPase reaction was functionally reversed to an ATP-synthase process in the course of evolution (Fig. 2). The original function of the H^+-ATPase, the link of metabolism and transport, might have been taken over in aerobic prokaryonts by the electrogenic H^+-extruding respiratory chain and in eukaryonts by an electrogenic $(Na^+ + K^+)$-ATPase.

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REFERENCES

1 Riebeling, V., Thauer, R. K. and Jungermann, K. (1975) Eur. J. Biochem. 55, 445–453
2 Munoz, F., Freer, J. H., Ellar, D. J. and Salton, M. R. J. (1968) Biochim. Biophys. Acta 150, 531–533
3 Munoz, E., Salton, M. R. J., Ng, M. H. and Schor, M. T. (1969) Eur. J. Biochem. 7, 490–501
4 Ishida, M. and Mizuchima, S. (1969) J. Biochem. Tokyo 66, 33–43
5 Mirsky, R. and Barlow, V. (1971) Biochim. Biophys. Acta 241, 835–845
6 Rosenthal, S. L. and Matheson, A. (1973) Biochim. Biophys. Acta 318, 252–261
7 Hachimori, A., Muramatsu, N. and Nosoh, Y. (1971) Biochim. Biophys. Acta 206, 426–437
8 Gross, R. and Coles, N. W. (1968) J. Bacteriol. 95, 1322–1326
9 Evans, D. J. (1969) J. Bacteriol. 100, 914–922
10 Kobayashi, H. and Anraku, Y. (1971) J. Biochem. Tokyo 71, 387–399
11 Roisin, M. P. and Kepes, A. (1972) Biochim. Biophys. Acta 275, 333–346
12 Roisin, M. P. and Kepes, A. (1973) Biochim. Biophys. Acta 305, 249–259
13 Hafkenscheid, J. C. M. and Bonting, S. L. (1968) Biochim. Biophys. Acta 151, 204–211
14 Davies, P. L. and Bragg, P. D. (1972) Biochim. Biophys. Acta 266, 273–284

15 Carreira, J., Leal, J. A., Rojas, M. and Munoz, E. (1973) Biochim. Biophys. Acta 307, 541–556
16 Monteil, H., Schoun, J. and Guinard, M. (1974) Eur. J. Biochem. 41, 525–532
17 Drapeau, G. R. and MacLeod, R. A. (1963) J. Bacteriol. 85, 1413–1419
18 Hayaishi, M. and Uchida, R. (1965) Biochim. Biophys. Acta 110, 207–209
19 Marunouchi, T. and Mori, T. (1967) J. Biochem. Tokyo 62, 401–407
20 Abrams, A. (1965) J. Biol. Chem. 240, 3675–3681
21 Abrams, A. and Baron, C. (1968) Biochemistry 7, 501–507
22 Schnebli, H. P. and Abrams, A. (1970) J. Biol. Chem. 245, 1115–1121
23 Sokawa, Y. (1965) J. Biochem. Tokyo 57, 706–708
24 Cole, H. A. and Hughes, D. E. (1965) J. Gen. Microbiol. 40, 81–95
25 Whittenbury, R. (1964) J. Gen. Microbiol. 35, 13–26
26 Harold, F. M. and Papineau, D. (1972) J. Membrane Biol. 8, 45–62
27 Asghar, S. S., Levin, E. and Harold, F. M. (1973) J. Biol. Chem. 248, 5225–5233
28 Decker, K., Jungermann, K. and Thauer, R. K. (1970) Angew. Chem. 82, 153–173 (Angew. Chem. Int. Edn. 9, 138–158)
29 Lovenberg, W., Buchanan, B. B. and Rabinowitz, J. C. (1963) J. Biol. Chem. 241, 3899–3913
30 Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
31 Fiske, C. H. and SubbaRow, Y. (1925) J. Biol. Chem. 66, 375–400
32 Cohn, M. (1963) Biochemistry 2, 623–629
33 Scrutton, M. C. (1973) in Inorganic Biochemistry, (Eichhorn, G. E., ed.), Vol. 1, pp. 381–437, Elsevier, Amsterdam
34 Holloway, C. T., Roberton, A. M., Knight, E. G. and Beechey, R. B. (1966) Biochem. J. 100, 79 p
35 Bulos, B. and Racker, E. (1968) J. Biol. Chem. 243, 3891–3900
36 Harold, F. M. (1970) Adv. Microb. Physiol. 4, 45–104
37 Harold, F. M. and Baarda, J. R. (1969) J. Biol. Chem. 241, 2261–2268
38 Pullman, M. E., Penefsky, H. S., Datta, A. and Racker, E. (1960) J. Biol. Chem. 235, 3322–3336
39 Racker, E. (1967) Fed. Proc. 26, 1335–1340
40 Harold, F. M., Pavlasova, E. and Baarda, J. R. (1970) Biochim. Biophys. Acta 196, 235–244
41 Harold, F. M. (1972) Bacteriol. Rev. 36, 172–230