Purification and Properties of the ATPase of the Halophilic and Alkaliphilic Phototrophic Bacterium *Ectothiorhodospira halochloris*

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The membrane-bound ATPase of the halophilic and alkaliphilic phototrophic bacterium *Ectothiorhodospira halochloris* was solubilized by washing membranes with buffer of low ionic strength and purified by ion-exchange chromatography, ultrafiltration and gel filtration. The *M_* of the trypsin-activated enzyme as determined by gel chromatography was approximately 400000. Four subunits were found by SDS electrophoresis with apparent *M_* values of 58000, 53700, 46800 and 36900. The purified enzyme was cold labile, but was more stable at room-temperature and in the presence of *p*-aminobenzamidine. Both the membrane-bound and the solubilized enzyme were inhibited by DCCD after preincubation, but not by oligomycin. The ATPase was activated by bicarbonate and sulphite. NaCl was inhibitory at low concentrations. The results are discussed with respect to optimum growth conditions for *E. halochloris* and its cytoplasmic solute concentrations. Responses of the enzyme to salts are compared to those of ATPases from two other halophilic bacteria.

**INTRODUCTION**

Proton-translocating ATPases (*F*<sub>0</sub>*F*<sub>1</sub>-ATPases, ATP synthases) have been isolated from mitochondria, chloroplasts and many different bacteria. A well-studied example of such a *F*<sub>0</sub>*F*<sub>1</sub>-ATPase is the enzyme from *Escherichia coli*. The enzyme consists of two different protein moieties: the *F*<sub>0</sub>-moiety, which is localized in the lipid bilayer of the membrane, and the *F*<sub>1</sub>-moiety, which is attached to *F*<sub>0</sub> at the surface of the cytoplasmic membrane. The complete enzyme catalyses ATP synthesis and ATP hydrolysis and links the proton-extruding reactions of energy generating systems (e.g. respiratory and photosynthetic electron-transport chains) to ATP synthesis, whereas the solubilized *F*<sub>1</sub>-complex catalyses only ATP hydrolysis. There are numerous reports and review articles on this enzyme (e.g. Fillingame, 1980; Futai & Kanazawa, 1983; Godinot & Di Pietro, 1986; Schneider & Altendorf, 1987).

The ATPases of two halophilic bacteria, the borderline moderately halophilic eubacterium *Vibrio costicola* (optimum growth at about 1 M-NaCl; Hipkiss *et al.*, 1980) and of the extremely halophilic archaeobacterium *Halobacterium saccharovorum* (optimum growth at 3-4 to 4-2 M-NaCl; Tomlinson & Hochstein, 1972) are halophilic enzymes (Cazzulo, 1978; Kristjansson & Hochstein, 1985). Optimum growth conditions for the borderline extremely halophilic phototrophic bacterium *Ectothiorhodospira halochloris* are a salinity of about 2.5 M-NaCl and pH 9.0 (Imhoff & Trüper, 1977). Successful adaptation to these growth conditions has made *E. halochloris* an interesting organism for the study of energy generation and ATP synthesis. In the present paper, we report on the solubilization, purification and some properties of the membrane-bound ATPase of *E. halochloris*.
METHODS

Culture conditions. Ectothiorhodospira halochloris (ATCC 35916, BN 9850) is an isolate from alkaline soda lakes of the Wadi Natrun in Egypt (Imhoff & Trüper, 1977). Cultures were grown (40 °C, 12000 lx illumination) in the medium described by Imhoff (1988). This medium, contained in 1 litre screw-cap bottles, was inoculated with 20% (v/v) of a well-grown, late-exponential phase preculture (OD850 1.9 to 2.2).

Preparation of cell extracts. Cells were harvested in late-exponential growth by centrifugation at 11000 g for 10 min and stored at −20 °C. For preparation of membranes, frozen cells were thawed and suspended in 50 mM-Tris/HCl, pH 8.0 [3 ml buffer (g wet wt cells)−1]. The same buffer was used for all following experiments unless otherwise specified. The cells were broken by one passage through a French pressure cell at Tris/HCl, pH 8.0, and cell debris were removed by centrifugation at 110000 g for 20 min. Membranes were separated from the soluble proteins by centrifugation at 200000 g for 120 min. The membranes were resuspended in buffer, centrifuged at 200000 g, and finally resuspended in buffer to a concentration of 10 to 20 mg protein ml−1 (‘washed membranes’). Activation by trypsin was done before the second ultracentrifugation step. The temperature was kept between 0 and 4 °C during the whole procedure.

Enzyme assays and enzyme purification. ATPase activity was determined in Tris/HCl buffer containing 1 mM-MgCl2 and 2 mM-ATP (total volume 1 ml) at 40 °C. The reaction was initiated by the addition of ATPase and terminated after 1 to 5 min by the addition of 0.1 ml trichloroacetic acid (3 m). After removal of the precipitated protein by centrifugation, inorganic phosphate (Pi) was determined in the supernatant according to the method of Taussky & Shorr (1953). One unit of enzyme activity is defined as the amount of enzyme producing 1 μmol P, min−1 under the indicated assay conditions. To confirm that Pi was liberated from ATP alone, both ADP and Pi, were determined in experiments with washed membranes. For ADP determination the reaction was terminated by addition of 0.1 ml perchloric acid (3 m) and the assay mixture neutralized with KOH (3 m). After centrifugation 0.6 ml of supernatant were mixed with 0.4 ml of a solution containing 100 mM-Tris/HCl (pH 7.2), 125 mM-KCl, 25 mM-MgCl2, 2.5 mM-phosphoenolpyruvate, 0.6 mM-NADH and 6 units of lactate dehydrogenase (EC 1.1.1.27) ml−1. After addition of 6 units of pyruvate kinase the oxidation of NADH was measured by recording the absorbance at 365 nm; the amount of ADP in the sample was calculated from the amount of oxidized NADH.

Gel filtration with Sephacryl S-300 (Pharmacia) was done on a 2 × 80 cm column at a flow rate of 20 ml h−1 using Tris/HCl plus 6 mM-p-aminobenzamidine as elution buffer. For ion-exchange chromatography, a DE-52 cellulose (Whatman) column (2 × 8 cm) was used at a constant pressure (60 cm water column). After loading the sample onto the column and washing with two bed-volumes of buffer the enzyme was eluted with a linear gradient of Tris/HCl buffer (50 mM, pH 8.0 to 500 mM, pH 7.2; total volume 200 ml).

For ultrafiltration, Diaflo XM 300 membranes were used in an Amicon ultrafiltration chamber with N2 as the pressurizing gas.

Other methods. SDS-gel electrophoresis was done according to Laemmli (1970) with 10% (w/v) acrylamide in the separation gel. Dissociation into subunits for SDS electrophoresis was achieved by heating samples to 100 °C for 2 min in sample buffer (Laemmli, 1970). Gels were stained with 0.02% Coomassie Brilliant Blue R250 in a mixture of methanol/acetic acid/water (1:0.2:0.8, by vol.) and destained with 10% (v/v) acetic acid. Phosphorylase b from rabbit muscle, bovine serum albumin, ovalbumin from egg white, carbonic anhydrase from bovine erythrocytes, trypsin inhibitor from soybean and α-lactalbumin from bovine milk (Pharmacia electrophoresis calibration kit) were used as M, standards. For densitometric evaluation of the gels a Hirschmann Elscript 400 densitometer was used.

Protein was determined by the biuret method (Beisenherz et al., 1953) for samples containing more than 1 mg protein ml−1 and by the method of Bradford (1976) for samples containing less protein.

For electron microscopy the purified enzyme was stained for 1 min with 1% (w/v) aqueous uranyl acetate (pH 4.5) and examined using a Siemens Elmiskop 101 electron microscope.

When ATP concentrations in the enzyme assay were varied, the Mg : ATP ratio of 1 : 2 was maintained. The actual MgATP concentrations were calculated according to Ahlers & Günter (1975) using a dissociation constant for MgATP of 0.215 mM (Wolf & Adolph, 1969).

Chemicals for electrophoresis were purchased from Serva, biochemicals from Boehringer; all other chemicals were of analytical grade from Merck.

RESULTS

Solubilization of the ATPase

The ATPase of E. halochloris is weakly bound to the membrane and can be easily solubilized by washing cell membranes in buffers of low ionic strength. With decreasing ionic strength the amount of ATPase solubilized increased, although the specific activity of the soluble protein fraction decreased in parallel. When membranes were suspended in 250 mM buffer at 0 °C, not more than 20% of the total ATPase activity was found in the supernatant. In 50 mM buffer about
ATPase from Ectothiorhodospira halochloris

Table 1. Purification of the ATPase from E. halochloris

Values refer to a purification protocol with all the steps done at 4 °C. Higher yields and specific activities were obtained when steps D to E were done at room-temperature and in the presence of p-aminobenzamidine.

<table>
<thead>
<tr>
<th>Protein fraction*</th>
<th>Specific activity <a href="%E2%88%921">units (mg protein)</a></th>
<th>Activity (units ml(−1))</th>
<th>Total activity (units per fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0-31</td>
<td>6-262</td>
<td>209-8</td>
</tr>
<tr>
<td>B</td>
<td>0-50</td>
<td>5-800</td>
<td>191-4</td>
</tr>
<tr>
<td>C</td>
<td>6-60</td>
<td>1-874</td>
<td>54-3</td>
</tr>
<tr>
<td>D</td>
<td>13-00</td>
<td>0-598</td>
<td>15-5</td>
</tr>
<tr>
<td>E</td>
<td>25-50</td>
<td>5-763</td>
<td>11-5</td>
</tr>
<tr>
<td>F</td>
<td>86-90</td>
<td>0-139</td>
<td>2-4</td>
</tr>
</tbody>
</table>

* A, trypsin-treated membranes; B, solubilized enzyme prior to separation from membranes; C, as B but after separation from membranes; D, eluate of DE-52 cellulose column; E, after ultrafiltration; F, eluate from Sephacryl S-300 column.

40% and in 5 mM buffer about 70% of the total ATPase activity was solubilized. The specific activity of the supernatant was 0-5, 0-75 and 1-5 units (mg protein)(−1) in 5, 50 and 250 mM buffer, respectively. Solubilization of the ATPase was not affected by the addition of 1 mM-MgCl₂, 0-5 mM-EDTA or 1 M-glycine betaine. Addition of 10% (v/v) glycerol increased the rate of solubilization, but due to inhibitory effects of glycerol the total solubilized activity was not increased. The ATPase could, however, be solubilized at a high specific activity by heating pretreated membranes to 40 °C (Table 1). Neither trypsin nor p-aminobenzamidine affected the rate of solubilization.

Latency of ATPase activity

The ATPase activity of washed membranes was approximately 50 to 60 mU (mg protein)(−1); this increased to 100 to 120 mU (mg protein)(−1) during overnight storage at 0 °C. Activation could be increased (1-2-fold with the solubilized enzyme and 1-7-fold with the membrane-bound enzyme) by heating to 30 °C for 30 min without any further treatment. Maximum stimulation of ATPase activity (up to 5-fold with washed membranes and about 2-fold with the solubilized enzyme) was achieved by treatment with trypsin at 30 °C. Maximum activation was found with 0-1 mg trypsin (mg protein)(−1) after 10 min at 30 °C. The reaction was terminated by the addition of trypsin inhibitor from hen egg-white (3 mg per mg of trypsin).

Purification

A typical purification protocol is shown in Table 1. Membranes of E. halochloris (pellet from the first ultracentrifugation) were resuspended in 50 mM buffer and activated by trypsin treatment as indicated above (fraction A in Table 1). Protein solubilized during this procedure was discarded after a second ultracentrifugation step. The resuspended membranes were heated to 40 °C for 10 min (fraction B). After another ultracentrifugation step the solubilized ATPase (supernatant; fraction C) was loaded onto a DE-52 cellulose column and eluted as described in Methods. The ATPase-containing fractions (fraction D) were concentrated by ultrafiltration (fraction E) and further purified by gel filtration with a Sephacryl S-300 column (fraction F). By this purification procedure the ATPase was normally purified 250- to 400-fold, as compared to trypsin-activated membranes (fraction A). When column chromatography and ultrafiltration were done at room-temperature and in the presence of 6 mM-p-aminobenzamidine during elution of the Sephacryl column, higher values (up to 700-fold) were obtained (data not shown). While only one band was obtained with this purified enzyme in native gel electrophoresis (1 µg protein per lane), minor impurities were detected by the more-sensitive SDS-gel electrophoresis (Fig. 1).
Area (%)...44.5 33.7 10.8 11.0

Fig. 1. SDS-gel electrophoresis of purified F$_{1}$-ATPase. Densitogram of a 10\% (w/v) acrylamide gel stained with Coomassie Brilliant Blue R250.

<table>
<thead>
<tr>
<th>[NaCl], [Na$_2$CO$_3$] (mM)</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>160</td>
</tr>
<tr>
<td>200</td>
<td>140</td>
</tr>
<tr>
<td>300</td>
<td>120</td>
</tr>
<tr>
<td>400</td>
<td>100</td>
</tr>
<tr>
<td>500</td>
<td>80</td>
</tr>
</tbody>
</table>

Fig. 2. Inhibition of the solubilized (○) and the membrane-bound (●) ATPase by sodium salts (a) and potassium salts (b). —— NaCl and KCl; —— Na$_2$CO$_3$ and K$_2$CO$_3$, pH 8.5. All values are means of duplicate determinations with deviations of less than 5%.

**M$_r$ and subunit composition**

An M$_r$ of approximately 400000 was determined by gel filtration using Sephacryl S-300. Four subunits were detected by SDS-gel electrophoresis; their M$_r$ values were 58000, 53700, 46800 and 36900 (mean values for three different enzyme preparations). The subunit composition of the ATPase was determined by densitometric evaluation of Coomassie-stained SDS-gels, which is, according to Foster & Fillingame (1982), a good measure of the molar ratio of the subunits. From the peak area and the M$_r$ values of the subunits a subunit ratio of 3:3:2:7:1:0:1:3 was calculated.

Electron microscopy of the purified enzyme revealed a hexagonal or ring structure similar to structures of F$_{1}$-ATPases from mitochondria (Kagawa & Racker, 1966), Alcaligenes faecalis (Adolfsen & Moudrianakis, 1971) and Micrococcus lysodeikticus (Nieto et al., 1975). With catalase crystals from bovine liver as a calibration standard (Ferrier & Murray, 1966), a diameter of 10 nm was determined for the ATPase of *E. halochloris*.

**Salt sensitivity**

The ATPase of *E. halochloris* was inhibited by both NaCl and KCl. Inhibition of the solubilized enzyme by NaCl was much greater than by KCl. Only 60\% of the control activity (without added salts) was observed at 50 to 100 mM-NaCl. In comparison, activity in the presence of 100 to 150 mM-KCl was more than 90\% of the control activity, and slowly dropped to 60\% at approximately 500 mM-KCl. The membrane-bound enzyme behaved somewhat differently (Fig. 2). Glycine betaine (1 M), the major compatible solute in *E. halochloris* (Galinski & Trüper, 1982), did not protect the solubilized enzyme against salt inhibition. Similarly, bovine serum albumin (5 mg ml$^{-1}$) had no effect on inhibition by salt.

Inhibition by NaCl was reversible: the same activity was observed when solubilized enzyme or membranes were preincubated with 500 mM-NaCl (1 h at 0 °C) and NaCl was diluted to 25 mM in the test assay as when the enzyme was assayed at 25 mM-NaCl without preincubation.

In contrast to the chlorides, both sodium carbonate (pH 8.5) and potassium carbonate
(pH 8.5) stimulated both membrane-bound and solubilized ATPase activity, showing maximum activity at 50 mM (Fig. 2). While stimulation by sodium carbonate was observed up to approximately 120 to 150 mM, potassium carbonate stimulated the solubilized enzyme up to a concentration of 500 mM.

** Catalytic properties**

In contrast to the membrane-bound and the solubilized enzyme, the purified ATPase was cold-labile and lost activity completely and irreversibly within 5 h when stored at 0 °C. At room-temperature, 50% of activity was lost within 5 h, when the enzyme was stored in buffer without any additives. However, more than 50% of enzyme activity was retained after 70 h at room-temperature, and also at 0 °C, when 6 mM-p-aminobenzamidine was added to the buffer. In the presence of 1 M-glycine betaine as a stabilizing agent, 50% of the initial activity remained after 50 h at room-temperature, but only 5% at 0 °C.

The ATPase of *E. halochloris* required magnesium for activity with an optimum Mg:ATP ratio of 1:2. Addition of 10 mM-EDTA inhibited activity completely; free Mg²⁺ ions were also strongly inhibitory. At an ATP concentration of 2 mM and various concentrations of magnesium, optimum activity was found at 1 mM-magnesium, but only 60% of this activity was observed at 2 mM-magnesium. This suggests that the MgATP-complex is the substrate. Inhibition of ATP hydrolysis in membrane preparations of *E. halochloris* by higher concentrations of Mg²⁺ excludes the existence of a membrane-bound 5'-nucleotidase as found in *Vibrio costicola*. This enzyme requires high magnesium concentrations and is most active at 20 to 50 mM-magnesium (Bengis-Garber & Kushner, 1981).

The ATPase of *E. halochloris* was not active when magnesium was replaced by calcium. Compared to the activity in the presence of magnesium, activity of the purified enzyme with calcium was only 16%. Maximum activity was found at a calcium:ATP ratio of 3:2. The membrane-bound enzyme showed no activity at all with Ca²⁺.

The optimum pH for activity of the purified enzyme was pH 8.0 in 50 mM-Tris/HCl but pH 8.5 in 50 mM-sodium carbonate buffer (58% activity at pH 8.0, 100% at pH 8.5, and 95% at pH 9.0). A flat temperature-optimum showed more than 90% of maximum activity between 40 and 53 °C with an absolute maximum near 50 °C. These values indicate optimum activity under optimum growth conditions for *E. halochloris*.

Oligomycin (5 µg ml⁻¹), which inhibits F₅F₇-ATPases by binding to F₅, had no effect on the membrane-bound ATPase activity of *E. halochloris*, although membrane-bound ATPase of *Rhodospirillum rubrum* was completely inhibited at this concentration (Müller & Baltzcheffsky, 1979). Inhibition of ATPase activity by DCCD in membrane fractions of *E. halochloris* was approximately 30% when membranes were preincubated for 30 min at 30 °C in buffer containing 0.1 mM-DCCD. Under the same conditions, activity of the solubilized ATPase was inhibited by 60%. (Both DCCD and oligomycin were added to the assay as stock solutions in 98%, v/v, ethanol; the ethanol concentration in the assay did not exceed 2% and had no effect on ATPase activity.)

For kinetic studies the substrate concentration was varied, while the Mg:ATP ratio was kept constant at 1:2. The MgATP concentrations were calculated according to Ahlers & Günther (1975). Under these conditions the *Kₘ* for MgATP was between 0.15 and 0.7 mM, depending on the storage time of the enzyme; *V* was 250 and 320 µmol Pi·min⁻¹·(mg protein)⁻¹ with freshly prepared ATPase and after storage for 24 h at room-temperature and in the presence of 6 mM-p-aminobenzamidine, respectively.

Bicarbonate and sulphite ions activated the purified ATPase at concentrations up to 200 mM. Activation by sulphite had a clear optimum at 10 mM, whereas activation by bicarbonate had a broad optimum between 10 and 200 mM (data not shown). Activation by both of these anions was due to increases in *V*, though they also caused an increase in the *Kₘ* for MgATP (Table 2). ATP hydrolysis was inhibited non-competitively by azide and competitively by ADP. The latter inhibition was partly overcome by the addition of bicarbonate and sulphite, indicating that activation by both anions is due to facilitation of the release of ADP from the enzyme. Table 2 shows the influence of some effectors on *Kₘ* and *V* values.
Table 2. Influence of various effectors on $K_m$ and $V$ values of the purified ATPase from *E. halochloris*

The recorded $K_m$ and $V$ values are mean values of triplicate determinations. Different control values without effectors are due to different storage times for the enzyme. The concentration of the MgATP complex was taken as the substrate concentration.

<table>
<thead>
<tr>
<th>Effector</th>
<th>$V$ [µmol P, min$^{-1}$ (mg protein)$^{-1}$]</th>
<th>$K_m$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>252</td>
<td>0.16</td>
</tr>
<tr>
<td>50 mM-NaHCO$_3$</td>
<td>364</td>
<td>0.50</td>
</tr>
<tr>
<td>100 mM-NaHCO$_3$</td>
<td>400</td>
<td>0.57</td>
</tr>
<tr>
<td>0.1 mM-Na$_2$SO$_3$</td>
<td>196</td>
<td>0.59</td>
</tr>
<tr>
<td>None</td>
<td>323</td>
<td>0.65</td>
</tr>
<tr>
<td>1 mM-ADP</td>
<td>323</td>
<td>1.96</td>
</tr>
<tr>
<td>1 mM-ADP + 100 mM-NaHCO$_3$</td>
<td>323</td>
<td>1.25</td>
</tr>
<tr>
<td>1 mM-ADP + 100 mM-Na$_2$SO$_3$</td>
<td>323</td>
<td>1.25</td>
</tr>
<tr>
<td>50 mM-Na$_2$SO$_3$</td>
<td>357</td>
<td>0.80</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Many properties, like $M_t$ and subunit composition, latency, cold lability, activation by sulphite and bicarbonate, and other catalytic properties of the ATPase of *E. halochloris* are similar to eubacterial F$_1$-ATPases. The F$_1$-ATPase of *Escherichia coli*, which can be regarded as typical of the proton-translocating F$_0$F$_1$-ATPases from bacteria, mitochondria and chloroplasts, comprises five different subunits with approximate $M_t$ values of 55000, 50000, 31000, 19000 and 15000 in a ratio of 3 : 3 : 1 : 1 : 1 (Futai *et al.*, 1974; Foster & Fillingame, 1982; Futai & Kanazawa, 1983). We have found that the ATPase of *E. halochloris* comprises four subunits, with approximate $M_t$ values for 58000, 54000, 47000 and 37000 in a ratio of 3 : 3 : 1 : 1. In several bacteria, the smallest subunit appears to be a natural inhibitor of the ATPase, which causes latent activity and is lost during activation. Because the ATPase of *E. halochloris* showed latent activity and was activated by trypsin prior to analysis of subunit composition, a fifth, inhibitory, subunit was probably lost during the purification procedures. We consider, therefore, that the ATPase of *E. halochloris* is an example of a highly evolved bacterial F$_1$-ATPase, which is part of a F$_0$F$_1$-complex (according to Harris, 1981). An F$_1$-ATPase from *Clostridium thermoaceticum* has been described that has four subunits, also in a ratio of 3 : 3 : 1 : 1 (Ivey & Ljungdahl, 1986). This enzyme could be activated neither by heat nor by trypsin treatment.

Remarkable properties of the ATPase of *E. halochloris* are the apparent lack of inhibition of the membrane-bound enzyme by oligomycin and DCCD and its easy separation from the membranes. The weakly bound F$_1$-ATPase was easily solubilized with low ionic strength buffers. As addition of neither EDTA nor MgCl$_2$ had a significant influence on its solubilization, the participation of divalent cations in its binding to the membrane can be excluded. As indicated by the effects of ionic strength and of glycerol on its solubilization, it is likely that the F$_1$-ATPase is bound to F$_0$ mainly by hydrophobic interactions.

Inhibition of the F$_1$-ATPase of the moderately to extremely halophilic (borderline extremely halophilic; see Kushner, 1978; Imhoff, 1986) *E. halochloris* at low salt concentrations contrasts with observations for F$_1$-ATPases from two other halophilic bacteria. The enzymes from the borderline moderately halophilic eubacterium *Vibrio costicola* (Cazzulo, 1978) and from the extremely halophilic archaeobacterium *Halobacterium saccharovorum* (Kristjansson & Hochstein, 1985) are clearly salt-dependent. The ATPase of *H. saccharovorum* requires 3.5 M-NaCl or KCl, while the enzyme of *V. costicola* is most active at 0.5 M-NaCl or KCl. As F$_1$ is located at the cytoplasmic side of the membrane, different salt responses of the three ATPases could reflect different cytoplasmic solute concentrations. In the cytoplasm of halobacteria molar concentrations of KCl are accumulated (see Kushner, 1978, for a review); elevated concentrations of salts have also been found in *V. costicola* (Shindler *et al.*, 1977), the major part of which appears, however, to be bound to cell structures and not freely dissolved in the...
cytoplasm (T. Ditandy & J. F. Imhoff, unpublished results). V. costicola also accumulates high concentrations of organic compatible solutes (Imhoff & Rodriguez-Valera, 1984). E. halochloris requires considerably higher salt concentrations for optimum growth than V. costicola (about 2-5 M compared to about 1 M-NaCl). Like V. costicola, it accumulates organic compatible solutes (glycine betaine, trehalose and ectoin) to achieve osmotic balance (Galinski & Trüper, 1982; Galinski et al., 1985). Its cytoplasmic salt concentration, in particular the cytoplasmic sodium concentration, is apparently maintained at a relatively low level. Although this has not yet been shown for E. halochloris, there are indications that the cytoplasm of other Ectothiorhodospira species is of low ionic strength (Imhoff & Riedel, 1989).

Since a key enzyme like the proton-translocating ATPase must require optimum conditions for activity in a growing cell, inhibition of the ATPase by low concentrations of NaCl and the failure of glycine betaine to overcome this inhibition suggest that E. halochloris maintains a low cytoplasmic concentration of NaCl. Inhibition by NaCl has also been found for other cytoplasmic enzymes of Ectothiorhodospira, including ribulose bisphosphate carboxylase of E. halophila (Tabita & McFadden, 1976) and malate dehydrogenase of several Ectothiorhodospira species, including E. halochloris (J. F. Imhoff, unpublished results). K+ and CO3 2- ions, however, are compatible with activity of the ATPase and are the only inorganic ions that could be accumulated in the cytoplasm up to 500 mM without inhibition of the ATPase.

With respect to growth temperature and pH, the ATPase of E. halochloris is apparently well-adapted to work under optimum growth conditions. Thus, optimum growth of E. halochloris is found between 40 and 50 °C and the optimum pH is about 9-0 (Imhoff & Trüper, 1977), although the cytoplasmic pH is maintained close to 8.5 under these conditions (J. F. Imhoff, unpublished results).

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REFERENCES


