Cation-activated Nucleotidase in Cell Envelopes of a Marine Bacterium

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Isolated cell envelopes of a marine bacterium, M.B.3, have been prepared which possess a nonspecific, cation-activated nucleotidase. The cell envelope comprises approximately 35% (dry weight) of the whole cell and contains protein, 60.2%; lipids, 20.7%; hexose, 3.4%; and ribonucleic acid, 4.6%. No deoxyribonucleic acid could be detected in the preparations. The nucleotidase has an essential requirement for Mg++; maximum activation at pH 8.0 occurs at a divalent cation concentration of approximately 80 mM. At a Mg++ to adenosine 5'-triphosphate (ATP) ratio of 2:1, the enzyme was further stimulated by monovalent cations Na+, K+, NH4+, and Li+. Maximum activity was found at a monovalent ion concentration of approximately 0.3 M. The envelope preparation liberated inorganic orthophosphate (P_i) from ATP, adenosine 5'-diphosphate (ADP), and adenosine 5'-monophosphate (AMP) at similar rates. Thin-layer and ion-exchange chromatography show that when AMP, ADP, and ATP were utilized as substrate, approximately 1, 2, and 3 moles of P_i, respectively, were produced per mole of adenosine. P_i was also liberated from the 5'-triphosphates of guanosine, uridine, and cytidine. The enzyme preparation did not attack p-nitrophenyl phosphate, β-glycerophosphate, or inorganic pyrophosphate. Sulfhydryl inhibitors p-chloromercuribenzoate, N-ethyl maleimide, and iodoacetate had little effect upon the nucleotidase activity. Ca++ and ethylene-diaminetetraacetic acid caused complete inhibition of the system, whereas ouabain had no effect upon the enzyme activity. The concentrations of Na+ (0.3 M) and Mg++ ions (60 to 80 mM) required for maximum ATP-hydrolyzing activity were similar to those concentrations necessary for maintenance of cell integrity and for the prevention of cell lysis.

Commmencing with the classical studies of Skou (26) on crab nerve preparations, numerous reports have appeared in the literature of adenosine 5'-triphosphate (ATP)-hydrolyzing systems in membranes of mammalian tissues. The ATPases (EC 3.6.1.4) are ubiquitously distributed (22, 27), and, being Mg++-dependent, some are also further stimulated by a combination of Na+ and K+ ions. There is an impressive amount of evidence to support the hypothesis that the ATP phosphohydrolase complex in mammalian systems, which is activated by a combination of Na+ and K+, is also involved in the active transport of Na+ and K+ ions against electrochemical gradients (28, 31).

ATP-hydrolyzing systems have been found in cytoplasmic membrane and cell envelope preparations of both terrestrial (1, 2, 12, 13, 19) and marine bacteria (9, 14). The ATPases from both groups of bacteria require Mg++ and can be further stimulated to varying extents by monovalent cations. Enzyme stimulation by Na+ and K+ is considerably less specific than in mammalian systems, and ouabain, a potent inhibitor of mammalian ATPase activity (7, 11), is without effect on bacterial systems. There is little direct evidence to suggest involvement of the ATP-hydrolyzing system in bacterial envelopes with Na+ or K+ cation transport across the cytoplasmic membrane. However, Solomon (29) suggested that the ATP phosphohydrolase in Escherichia coli cell membranes may be involved with K+--H+ exchange in this organism.

There appears to be a major difference between the enzymatic action of the ATP-hydrolyzing system in terrestrial and marine bacterial species. The ATPase in envelopes of terrestrial organisms Streplococcus faecalis (1, 2), Staphylococcus aureus (13), and Bacillus megaterium (12, 30) appears to be a true ATPase insofar as products of ATP hydrolysis are ADP and P_i as in mammalian...
systems. The two reports of ATPase activity in envelopes of marine or halophilic bacteria by Drapeau and MacLeod (9) and Hayashi and Uchida (14) show that adenosine 5'-monophosphate (AMP) and adenosine 5'-diphosphate (ADP) can also be utilized as substrates.

The properties of an ATP phosphohydrolase in cell envelopes of a marine bacterium, M.B.3, have been studied to determine if nucleotidase rather than ATPase activity is a characteristic feature of halophilic cell envelopes. The possible relationships between cation requirement for maintenance of cell integrity and maximum stimulation of enzymatic activity have also been investigated.

**MATERIALS AND METHODS**

**Maintenance of stock culture.** The organism used in these studies, a gram-negative marine bacterium designated M.B.3, was obtained from M. E. Tyler, Department of Bacteriology, University of Florida, Gainesville, Florida. The bacterium was maintained by monthly transfer on slants of a medium containing 1% Difco Special Agar (Noble), in a solution containing (per liter): NaCl, 24 g; KCl, 0.7 g; CaCl2, 1.0 g; MgSO4·7H2O, 5.2 g; MgCl2·6H2O, 6.9 g; and Casamino Acids, 5.0 g. The mixture was adjusted to pH 7.4 with sodium hydroxide. The medium was sterilized in the autoclave at 15 psi for 20 min (Mg2+ salts were sterilized separately).

**Growth of cells.** The organism was grown in medium of the above composition with agar omitted. Normally, 5 liters of sterile growth medium were inoculated with 50 ml of fresh cell suspensions. The culture was aerated at 30 C for 17 hr prior to harvesting on the de Laval centrifuge. The resulting cell paste was subsequently washed three times by resuspension in, and centrifugation (16,000 × g for 15 min) from, 250-ml volumes of the basal salts solution described above.

**Preparation of cell envelopes.** Approximately 10 g (wet weight) of cells were suspended in 20 ml of 0.001 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (pH 8.0). The suspension was then poured into 500 ml of the same buffer when almost instantaneous lysis of the cells occurred. Deoxyribonuclease and ribonuclease at a final concentration of 20 µg/ml were added to the lysed cell suspension. The mixture was stirred for 30 min and then centrifuged at 54,000 × g for 20 min at 4 C. The supernatant fluid was discarded, and the envelope pellet was washed in 0.001 M Tris-hydrochloride buffer (pH 8.0) until no material absorbing at 260 to 280 nm could be detected in the supernatant fluid. The “clean” envelope pellet was then resuspended in 3 parts (w/v) 0.01 M Tris-hydrochloride buffer (pH 8.0) and sonically treated 3 min at 1 amp with a Dawes Soniprobe. After sonic disruption, the suspension was then centrifuged at 54,000 × g for 30 min. The supernatant fluid, which was essentially a solubilized membrane preparation, was removed and utilized for the enzyme studies.

**Analytical methods.** Protein was determined by the biuret modification of Itzhaki and Gill (15) with bovine serum albumin as standard. Total protein of envelopes was also estimated by the micro-Kjeldahl and Nessler methods by determination of total nitrogen. Hexose was measured by the anthrone method of Bartnicki-Garcia and Nickerson (3), inorganic orthophosphate (P) by the Gomori modification of the Fiske-SubbaRow method (10). Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) in trichloroacetic acid-extracted (25) envelopes of M.B.3 were estimated by the orcinol method (20). Total lipid was determined by the method of Salton (24).

Adenosine and adenylic nucleotides were assayed spectrophotometrically at 257 nm with the Unicam S.P. 500 spectrophotometer.

**Thin-layer and ion exchange chromatography.** Nucleotide reaction products were qualitatively separated and identified on thin-layer plates of diethyl aminomethyl cellulose. The method was based on that described by Randerath (23) and, for analysis, 20-µlitter-samples of the reaction system were applied to the plates. The plates were first developed in distilled water. This process resulted in the separation of adenosine, which moves just behind the water front, from the nucleotides, which remained at the point of application. It was also found that prior development of the plate by this method minimized streaking due to the presence of salts. The plates were air-dried and placed in the second solvent, 0.02 N HCl. This solvent, which was run in the same direction as before, gave satisfactory resolution of the nucleotides in the sample. Development time was approximately 1 hr in both cases. The nucleotides were detected under shortwave ultraviolet light.

Adenosine and adenylic nucleotides in the reaction systems were assayed quantitatively by a modification of the method of Cohn and Carter (8), in which 0.5 ml of incubation medium was pipetted onto the surface of a Dowex 1 × 8 Cl- ion-exchange column (1 by 1 cm). Adenosine was eluted with distilled water, AMP with 0.01 N HCl, ADP with 0.02 M NaCl in 0.02 N HCl, and ATP with 0.20 M NaCl in 0.02 N HCl. The various eluent fractions were pooled, and total nucleotide present was estimated spectrophotometrically at 257 nm. Recoveries of 100 ± 5% total nucleotide could be attained.

**Electron microscopy.** Washed cell envelopes in distilled water were embedded in collodion on 3.05-mm copper grids. The specimens were coated with carbon and shadowed with chromium evaporated from a tungsten coil in vacuo. The preparation was examined with the A.E.I. electron microscope EM 6B at 80 kv.

**Measurement of enzyme activity.** Unless otherwise specified, the standard assay system contained 2.5 mm ATP-free acid (Tris-neutralized), 5 mm MgCl2, and 0.05 M Tris hydrochloride buffer (pH 8.0). The concentration of envelope preparation varied from 1 to 5 mg of protein in the final assay volume of 3 ml. In the cation-activation studies, salts in the chloride form were added to give the desired final concentration. Incubation was usually carried out at 25 C for 15 min. Enzymatic activity was halted by the addition of 1.0 ml of 10% (w/v) trichloroacetic acid to the system. [In thin-layer studies the reactions were stopped by addition of ethylenediaminetetraacetic acid (EDTA).]
Precipitated protein was removed by centrifugation, and 1.0 ml of the supernatant fluid was assayed for Pi. Suitable corrections were made for nonenzymatically liberated Pi. Specific activity of the enzyme preparation was expressed as micromoles of Pi liberated per milligram of protein per minute.

Studies on lysis of M.B.3. A 10-g (wet weight) pellet of M.B.3 was suspended in 20 ml of complete salts solution. One milliliter of this cell suspension was transferred to 100 ml of the appropriate Mg2+ or Na+ solution. The diluted suspensions were stirred for 30 min at 25 C, and 35-ml volumes of suspension were centrifuged at 25,000 X g for 20 min. The supernatant fluids were carefully decanted and the optical density (OD) (at 280 nm was recorded. With the OD value obtained when complete lysis of cells occurred in distilled water as 100%, the extent of cell lysis in the various salt solutions could be evaluated.

RESULTS

Cell envelope preparations. Figure 1 illustrates a typical envelope preparation of M.B.3 viewed under the electron microscope. The cell envelopes were relatively free from intracellular materials, although traces of nuclear-dense material, probably RNA, could be seen adhering to the inner surface in some preparations.

Chemical analysis of envelopes. The cell envelopes were found to comprise approximately 35% of the total dry weight of the whole cell. By the techniques described, the chemical composition was found to be: protein (by biuret) 60.2%; by (by total nitrogen) 66.9%; lipid, 20.7%; hexose, 3.4%; RNA, 4.6%. No DNA could be found in the preparations.

Localization of the ATP-hydrolyzing enzyme. A lysed cell preparation was obtained as described. A portion of the lysate was centrifuged at 54,000 X g for 30 min. The supernatant fluid was carefully removed, and the residual envelope pellet was resuspended in an amount of 0.01 M Tris-hydrochloride buffer (pH 8.0) equal to the volume of the supernatant fluid. Quantitative comparison of enzymatic activities in the three fractions—lysate, cell envelope, and intracellular cytoplasmic material—was thereby assured. The distribution of ATP-hydrolyzing enzyme is shown in Table 1. The results indicate that almost 96% of the total enzymatic activity was associated with the cell envelope fraction.

Optimum pH. Two buffer systems were used to determine the optimum pH of the ATP-

![Fig. 1. Electron micrograph of cell envelope preparation of M.B.3, prepared and mounted as described.](http://jb.asm.org/)
hydrolyzing system, 0.05 mM Tris-hydrochloride and 0.05 mM glycine-NaOH, which covered the pH ranges 7 to 9.0 and 8 to 10.5, respectively (Fig. 2). Enzymatic activity increased sharply with increasing pH until the pH optimum of 8.0 was reached. Higher pH resulted in a sharp decline in enzyme activity.

**Activation by Mg^{2+}.** Figure 3 shows the effect of increased Mg^{2+} concentration upon the activity of the ATP-hydrolyzing enzyme from a preparation of M.B.3 cell envelopes. The enzyme, in common with those ATPase systems from bacterial (1, 2, 9, 13, 30) and mammalian sources (26, 27), had an essential requirement for the divalent cation. Maximum activation occurred at Mg^{2+} concentrations approaching 80 mM. The results also show that AMP and ADP as well as ATP were hydrolyzed by the enzyme preparation and all three systems were activated similarly by Mg^{2+}.

**Influence of monovalent cations.** It was found that the activity of the Mg^{2+}-dependent enzyme preparation could be further increased by addition of any one of four monovalent cations. The effects of increasing concentrations of Na^+, K^+, Li^+, and NH_4^+ ions upon enzyme activity when the Mg to ATP ratio was 5 mM Mg^{2+}:2.5 mM ATP are illustrated in Fig. 4. The results of activation by Na^+ and K^+ or combinations of these ions are shown in Table 2. The enzyme was not preferentially activated by any one or a combination of cations. There was no further stimulation by monovalent cations when these were added to assay systems already containing 100 mM Mg^{2+}.

**Action of enzyme preparation upon ATP, ADP, and AMP.** The standard assay system in which AMP and ADP at 2.5 mM concentrations replaced the ATP was used to investigate the activity of the enzyme upon all three adenine nucleotides (Fig. 5). The preparation released Pi from all three nucleotides and, whereas Pi was liberated at approximately the same rate from AMP and ATP, hydrolysis of ADP occurred at a slightly faster rate.

**Table 1. Relative distribution of ATP-hydrolyzing enzyme in the marine bacterium M.B.3**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein mg</th>
<th>Total Pi liberated per 30 min</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysate (cell envelope + cytoplasm)</td>
<td>1.25</td>
<td>3.78</td>
<td>0.094</td>
</tr>
<tr>
<td>Cytoplasm</td>
<td>0.80</td>
<td>0.20</td>
<td>0.0083</td>
</tr>
<tr>
<td>Envelope</td>
<td>0.556</td>
<td>3.64</td>
<td>0.220</td>
</tr>
</tbody>
</table>

**Fig. 2.** Effect of pH upon the rate of release of inorganic phosphate (Pi) from ATP by cell envelope preparation of M.B.3. Symbols: , 0.05 mM Tris-hydrochloride; ○, 0.05 mM glycine-NaOH buffer.

**Fig. 3.** Effect of increased Mg^{2+} concentration upon the rate of release of Pi from adenine nucleotides by the cell envelope preparation. The 5-ml incubation systems contained 2.5 mM nucleotide, 0.05 mM Tris-hydrochloride buffer (pH 8.0), and 0.75 mg of protein. Incubation period was 15 min, and 1.0-ml portions were assayed for Pi as described.

**Thin-layer chromatography of reaction products on DEAE cellulose.** Qualitative analyses of the reaction products formed when the three adenine nucleotides were incubated with envelope preparations were performed by thin-layer chromatography. It was found originally that when ADP was used as substrate the intensity of the spots
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Fig. 4. Stimulation of the ATP-hydrolyzing enzyme by monovalent cations. Point A contains 2.5 mM ATP but no Mg²⁺; in B and all other points shown, the incubation medium contained 5 mM Mg²⁺ and 2.5 mM ATP.

Table 2. Stimulation of ATP-hydrolyzing enzyme activity by Na⁺ and K⁺, either separately or in combination, in presence of 2.5 mM Mg²⁺

<table>
<thead>
<tr>
<th>Cation concentration</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg²⁺ (alone, 2.5)</td>
<td>0.072</td>
</tr>
<tr>
<td>Mg²⁺ 2.5 in each assay system +</td>
<td>0.153</td>
</tr>
<tr>
<td>Na⁺ 100</td>
<td>0.220</td>
</tr>
<tr>
<td>Na⁺ 50 + K⁺ 150</td>
<td>0.207</td>
</tr>
<tr>
<td>Na⁺ 200</td>
<td>0.211</td>
</tr>
<tr>
<td>K⁺ 200</td>
<td>0.22</td>
</tr>
</tbody>
</table>

corresponding to AMP and ATP sometimes increased progressively through the incubation period. On other occasions, no such spots could be seen. Further investigation revealed the presence of adenylate kinase (EC 2.7.4.3) in cell envelope preparations which had been incompletely washed during the preparative stages. With "clean" envelope preparations, the end products of enzymatic action upon AMP, ADP, and ATP were adenosine and P₁. No intermediates could be detected in the ADP or ATP incubation systems.

Quantitative analysis of reaction products on Dowex 1 × 8 Cl⁻ resin. The results of quantitative analyses of the reaction systems throughout the incubation period are shown in Fig. 6. Irrespective of the nucleotide substrate, only adenosine and P₁ could be detected in the systems. The

Table 3. Substrate specificity of cell envelope preparation of marine bacterium M.B.3

<table>
<thead>
<tr>
<th>Nucleotide substrate (2.5 mM)</th>
<th>Per cent activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>100</td>
</tr>
<tr>
<td>GTP</td>
<td>114</td>
</tr>
<tr>
<td>UTP</td>
<td>56.8</td>
</tr>
<tr>
<td>CTP</td>
<td>35.2</td>
</tr>
</tbody>
</table>

* P₁ liberated in 15 min expressed as percentage of that liberated with ATP as substrate.

Fig. 5. Release of P₁ from AMP, ADP, and ATP by a cell envelope preparation.

Fig. 6. Quantitative analyses from ion-exchange chromatography of reaction products formed when M.B.3 cell envelope preparation was incubated with AMP, ADP, and ATP.
stoichiometric molar ratios of P1 to adenosine produced at any period of incubation of AMP, ADP, and ATP were found to be 0.98, 2.05, and 3.23, respectively.

**Specificity of cell envelope preparations.** The action of the ATP-hydrolyzing preparation upon guanosine 5'-triphosphate (GTP), cytidine 5'-triphosphate (CTP), and uridine 5'-triphosphate (UTP) was investigated. After 15 min of incubation, the P1 liberated from each nucleotide was compared to that liberated over a corresponding interval from an ATP-incubation system (Table 3). It is evident that P1 was liberated more quickly from GTP and more slowly from UTP and CTP than from ATP. Inorganic pyrophosphate, glucose-6-phosphate, β-glycerophosphate, and p-nitrophenyl phosphate at 2.5 mm concentrations were substituted for ATP in the incubation systems. No P1 was released from any of these compounds.

**Inhibitor studies.** Sulphydryl inhibitors iodoacetate, p-chloromercuribenzoate, and N-ethylmaleimide at 10⁻³ M and 10⁻² M had no inhibitory effect upon the activity of the enzyme. Similarly ouabain (γ-strophanthin), a potent inhibitor of ATPases from various mammalian systems, produced no inhibition of the ATP phosphohydrolase from envelope preparations of M.B.3. Ca²⁺ and EDTA, both well-known inhibitors of many Mg²⁺-dependent phosphatases, produced complete inhibition at a final concentration of 10⁻² M.

**Prevention of cell lysis by Mg²⁺ and Na⁺.** Preliminary studies indicated that, as the ionic concentration of the environment was reduced, the cells began to lyse. The relative capacities of mono- and divalent ions to prevent lysis were accordingly investigated (Fig. 7). It was found that divalent Mg²⁺ ion was much more effective than Na⁺ in preventing lysis and, whereas 80% of the cells remained intact with 60 mM Mg²⁺, a similar degree of integrity could only be achieved by approximately 220 mM Na⁺.

**DISCUSSION**

ATPase preparations from mammalian tissues (26, 27, 31) in the presence of Mg²⁺ hydrolyze ATP with the liberation of P1 and the formation of ADP in stoichiometric proportion. The preparations usually have a negligible effect upon AMP or ADP; indeed, ADP in some cases is a competitive inhibitor of enzyme activity. The ATPases from terrestrial bacterial envelopes [B. megaterium (12, 13), S. faecalis (1, 2), and S. aureus (13)] also appear to be ATPases in the accepted sense in that the products of ATP hydrolysis are ADP and P1.

The enzyme in isolated cell envelopes of the marine organism M.B.3 can liberate P1 from a variety of nucleotides. In addition to ATP, AMP, and ADP, which are attacked at similar rates, so too, to differing extents, are GTP, CTP, and UTP. Qualitative and quantitative analyses of the reaction products of enzyme action upon the three adenine nucleotides show free adenosine and P1 to be formed in all cases. The stoichiometric ratio between the amounts of P1 to adenosine produced when the three nucleotides AMP, ADP, and ATP were used as substrates was found to be 0.98, 2.05, and 3.23, respectively. Whereas the results do not allow definite conclusions to be drawn as to whether hydrolytic activity is due to one or more phosphohydrolases, the indications in favor of one enzyme are (i) P1 liberation from all three nucleotides occurring at approximately the same rate, (ii) the same Mg²⁺ activation patterns were observed irrespective of the adenine nucleotide utilized as substrate, (iii) the failure to detect intermediate reaction products in the ATP- and ADP-incubation systems by either thin-layer or ion-exchange chromatography. The enzyme closely resembles the ATP-hydrolyzing systems in cell envelopes of two other marine organisms, Vibrio paraaeromolyticus (14) and the pseudomonad B16 (9), which produce 3 moles of P1 per mole of ATP hydrolyzed. Though these preparations attack AMP and ADP, quantitative analysis of the reaction products was not undertaken.

The dependence of the phosphatase in M.B.3 envelope preparations upon Mg²⁺ for activity is in agreement with similar requirements for activation of ATPase in mammalian tissue and in terrestrial bacterial envelopes. The observation that the enzyme in M.B.3 preparations required far greater Mg²⁺ concentration for optimum activity than the latter systems is worthy of comment. In terrestrial bacteria and mammalian tissues, maximum stimulation of the ATPase usually occurs when the Mg²⁺ to ATP ratio is 1:1 or at most 2:1.
Considering together the pK values of the primary and secondary hydroxyl groups of the phosphate moieties and the fact that the pH optimum for the reaction is pH 8.0, it is concluded that in the assay system the ATP molecule will possess four negative charges. The combination of Mg$^{2+}$ and ATP$^-$ → [MgATP$^-$] was investigated by Burton (6) and O'Sullivan and Perrin (21), who demonstrated the equilibrium position to lie far to the right in this reaction. This observation would suggest that when Mg$^{2+}$ and ATP$^-$ are present in equimolar proportions in a system, little free cation or nucleotide exists. Equilibrium studies of the Mg$^{2+}$-ATP$^-$ reaction, together with the observation that maximal ATPase activity is found usually at Mg$^{2+}$-ATP ratios of unity, is strong evidence that MgATP$^-$ is the true substrate of this enzyme.

As previously noted, maximum activation of the ATP-hydrolyzing enzyme from M.B.3 envelopes occurs at Mg$^{2+}$ concentrations some 30 times in excess of the nucleotide concentration. Similar results were reported by Hayashi and Uchida (14) for the ATPase of V. parahaemolyticus which required 150 mm Mg$^{2+}$ (at a nucleotide concentration of 2 mm) for maximum activity. It would appear likely that, because of the equilibrium position of the Mg$^{2+}$-ATP$^-$ reaction, activation by such high Mg$^{2+}$ levels cannot simply be due to an increased concentration of the metallosubstrate MgATP$^-$ alone. In view of the nonspecific stimulation produced by monovalent cations Na$^+$, Li$^+$, K$^+$, and NH$_4^+$, it is suggested that the increased activity of the hydrolyzing enzyme in M.B.3 envelope preparations is due to an ion-protein rather than an ion-substrate interaction. Nonspecific monovalent cation stimulation of an ATP-hydrolyzing system from spheroplast preparations of a marine pseudomonad has also been reported by Drapeau and MacLeod (9).

The organism M.B.3, in common with almost all marine bacteria, lysed rapidly in distilled water or in dilute 0.001 m Tris-hydrochloride buffer. The evidence available suggests that the requirement for cations for prevention of lysis of marine bacteria is not simply an osmotic requirement (D. Pratt and W. Riley, Bacteriol. Proc., p. 26, 1955), since individual salts differ in their ability, at equivalent concentrations, to prevent cell lysis (18). MacLeod and Matula (17) have also shown that divalent cations such as Mg$^{2+}$ and Ca$^{2+}$ are more effective at much lower salt concentrations than monovalent cations in maintaining integrity of a marine pseudomonad. Current ideas of cation action favor a "polyelectrolyte" approach (16) in which cations are associated with negatively charged material in the outer layers of the cell. Other workers (5) have postulated that there is cationic involvement with a mucoprotide layer lying adjacent to the cytoplasmic membrane. It is also believed that this layer is composed of subunits which are able to come close enough together to form a continuous layer (and so maintain cell integrity) only if the negatively charged areas are screened by cations. Brown (4) postulated that cations may prevent lysis of the marine forms by preventing the action of cell wall-lytic enzymes.

The concentrations of Mg$^{2+}$ and Na$^+$ which prevent lysis of M.B.3 are approximately 60 mm and 300 to 400 mm, respectively, and these concentrations closely parallel the ion requirements of the ATP-hydrolyzing enzyme in envelope preparations for maximum activation. The results may indicate that, in this marine organism, only when the outer layers of the cell have an optimum cationic environment will the enzymes associated with this region have their optimum conformations and activities.

The ATP-hydrolyzing system in the marine bacterium M.B.3 was located solely in the envelope fraction, and ouabain, a potent inhibitor of Na$^+$-K$^+$-activated ATPases of mammalian systems, was without effect upon enzyme activity. EDTA and Ca$^{2+}$ produced complete inhibition of phosphatase activity. No evidence was obtained to suggest involvement of the enzyme with Na$^+$-K$^+$ transport in the halophile. On the basis of their wide specificity toward nucleotides, nonspecific monovalent cation activation, and on the basis of the reaction products, it would seem preferable to identify the ATP phosphohydrolase system in the three marine bacteria examined as a cation-activated nucleotidase rather than an ATPase. Whether such an ATP-hydrolyzing enzyme is a characteristic of marine bacteria must await future studies with other organisms.

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LITERATURE CITED


