ATP Hydrolysis in a Marine Bacterium

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The membrane-bound adenosine triphosphatase of marine pseudomonad B-16, when solubilized, is able to rebind to depleted membrane residues of the bacterium and to those of Escherichia coli.

Studies on membrane-bound Mg2+ adenosine triphosphatase (ATPase) aggregate in Escherichia coli and other bacteria have revealed the enzyme to be composed of two parts, a membrane-bound protein system (coded by the uncB gene) and another protein complex loosely attached to the membrane (coded by the uncA gene), both of which are composed of many protein-complex subunits (4). The former protein binds N,N′-dicyclohexyl carbodiimide (DCCD) (1) and is associated with proton permeability of the membrane (15). The other protein, which is easily leached from the membrane by mild treatments (9), possesses an ATP hydrolysis function but is not sensitive to DCCD (7). In several systems, an active ATPase aggregate attached to the membrane can synthesize ATP when a transient H+ gradient is formed (acid out) either artificially or by electron transport-driven proton exclusion (7, 13). It can also catalyze an ATP-dependent acidification of the medium that can drive active transport (10) or transhydrogenation reactions (8, 16).

Marine pseudomonad B-16, variant 3 (6), and an alkaline phosphatase (APase)-negative mutant were grown at 25°C in the complex medium to the late logarithmic phase, harvested, and washed in a complete salts solution as described before (12). E. coli (AN 259, a generous gift of P. Gibson, Australian National University, Canberra, Australia) was grown in nutrient broth supplemented with 1% glucose to late log phase and washed in 0.85% physiological saline. Cell-free extracts were obtained by the use of a French pressure cell. Particulate and soluble fractions were prepared by ultracentrifugation (12). Particles were washed once by ultracentrifugation to produce washed particles. In certain experiments, the washed particles were suspended in a small volume (20 mg of protein per ml) and dialyzed as described by others (9). The dialyzed particles, which were depleted of ATPase activity, were recovered by centrifugation as described before (12). The supernatant fraction of the dialysate, which contained the solubilized coupling factor or ATPase, was concentrated by freeze-drying.

The APase-negative mutant was isolated after ethyl methane sulfonate treatment that killed 99.9% of the population (11) by the method of Torriani and Rothman (18).

Cell-free extracts, washed particles, dialyzed particles, and the supernatant fraction, were screened for alkaline phosphatase (2), cyclic phosphodiesterase, ribonuclease (3), isocitrate dehydrogenase, cytochromes c549, c552, and b559 (12), alcohol dehydrogenase (17), and ATP hydrolysis activity in the presence of 1 mM cyanide and/or 0.1 mM DCCD (14). Protein was measured by a modified biuret reaction as described previously (12).

Polyacrylamide gel electrophoreses of various cell fractions of the parent and mutant marine pseudomonads and E. coli were run by the procedure of Davis (5). The resultant gels were cut into 2-mm sections and homogenized in 50 mM tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 8.0). Each fraction was assayed for APase (2) and ATP hydrolysis activity (14). ATP and DCCD were obtained from Sigma Chemical Co., St. Louis, Mo.

ATP hydrolysis, the usual assay for ATPase activity (14), gives a misleading measure of this enzyme because of the presence of an active alkaline phosphatase in marine pseudomonad B-16. Table 1 shows that ATP hydrolysis comprises two separate activities, one DCCD sensitive (ATPase) and the other cyanide sensitive (APase). For this reason, a mutant lacking APase activity was isolated. From Table 1 it is evident that this mutant not only lacks APase activity, but also lacks cyanide-sensitive ATP hydrolysis. This mutant shows normal activity for a variety of other enzyme functions, including cytoplasmic, membrane-bound, and periplasmic markers. This indicates that the mutation is most probably specific for APase and does not result in weakened wall structure. These data (Table 1) also indicate that ATP hydrolysis in the mutant is 84% sensitive to DCCD (a higher...
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Table 1. Enzyme activities of cell-free extracts of the wild type and APase mutant marine pseudomonad B-16

<table>
<thead>
<tr>
<th>Enzyme*</th>
<th>Wild type</th>
<th>APase negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>APase</td>
<td>0.029</td>
<td>0.003</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>0.015</td>
<td>0.018</td>
</tr>
<tr>
<td>Cyclic phosphodiesterase</td>
<td>0.0015</td>
<td>0.0011</td>
</tr>
<tr>
<td>Cytochrome c549</td>
<td>0.48</td>
<td>0.65</td>
</tr>
<tr>
<td>Cytochrome c562</td>
<td>0.65</td>
<td>0.84</td>
</tr>
<tr>
<td>Cytochrome b509</td>
<td>0.45</td>
<td>0.68</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>0.97</td>
<td>1.58</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>0.078</td>
<td>0.135</td>
</tr>
<tr>
<td>ATP hydrolysis</td>
<td>0.021</td>
<td>0.0124</td>
</tr>
<tr>
<td>+ 1 mM cyanide</td>
<td>0.010</td>
<td>0.0116</td>
</tr>
<tr>
<td>+ 0.1 mM DCCD</td>
<td>0.013</td>
<td>0.002</td>
</tr>
<tr>
<td>+ 1 mM cyanide + 0.1 mM DCCD</td>
<td>0.003</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* IU, 1 μmol of substrate consumed per min or, for cytochromes, 1 nmol of cytochrome.

All enzymes were assayed in cell-free extracts, except cytochrome c549, which was assayed in the soluble fraction, and cytochromes c562 and b509, which were assayed in the particulate fraction.

concentration of the inhibitor caused no further inhibition.

To further check that APase was responsible for ATP hydrolysis in the wild-type, cell-free extract, polyacrylamide gels of the wild-type and mutant extracts were run. These were cut up and assayed for APase and ATP hydrolysis activities. Figure 1 illustrates such an experiment. As can be seen, the wild type gives one peak for APase activity and two peaks for ATP hydrolysis activity. One peak is superimposable on the APase activity peak, whereas the other does not enter the gel. The mutant lacking the APase activity has also lost its ATP-hydrolyzing activity corresponding to the APase activity. The fraction of the activity not entering the gel is still present and is most probably the classical DCCD-sensitive Mg2+, APase.

Washed particles derived from the APase-negative mutant of marine pseudomonad B-16 and E. coli AN 259, a strain possessing normal ATPase structure and function, were examined (4). ATPase activities of these particles were at least 88% sensitive to DCCD (Table 2). However, when dialyzed, ATPase activity was released from both membrane sources and appeared in the supernatant fraction (Table 2). This activity, comprising at least 80% of the total activity, was no longer sensitive to DCCD (at most 10%). When solubilized from the membrane, the enzyme was capable of migrating into polyacrylamide gels, but the membrane-bound activity was unable to migrate (Fig. 2). However, if particles that had been depleted of ATPase activity and the supernatant fractions were mixed in complete salts-tris(hydroxymethyl)aminomethane buffer, reassociation of the ATPase with the membrane occurred, as evidenced by the appearance of DCCD sensitivity (Table 2) and the inability of the enzyme to migrate into particles.

![Table 1. Enzyme activities of cell-free extracts of the wild type and APase mutant marine pseudomonad B-16](http://jb.asm.org/)

![Table 2. DCCD sensitivity of E. coli and marine pseudomonad B-16 (APase negative) ATPase](http://jb.asm.org/)

![Figure 1. Fractionation of ATP hydrolysis activity in the wild type and in APase-negative mutant of marine pseudomonad B-16 by polyacrylamide gel electrophoresis. Approximately 0.2 mg of protein of cell-free extracts of the wild type (A) and an APase mutant (B) were applied to polyacrylamide gels and run for 30 min as described in the text. Gels were sliced into 2-mm fractions and assayed for APase (Δ) or ATP hydrolysis activity (○). Enzyme activity is expressed in international units (IU), which corresponds to the hydrolysis of 1 μmol of substrate per min. Fraction 0 is the buffer immediately above the gel; fraction 1 is the origin.](http://jb.asm.org/)
Fig. 2. Electrophoretic properties of ATPase activity of an ATPase-negative mutant of marine pseudomonad B-16 and E. coli AN 259. Approximately 0.2 mg of protein from depleted particles (Pd) and/or solubilized factor (Sp) of an ATPase mutant of marine pseudomonad B-16 or E. coli (AN 259) was applied to polyacrylamide gels and run for 30 min as described in the text. Gels were cut into 2-mm fractions and assayed for ATP hydrolysis activity. Enzyme activity is expressed in international units (IU). Fraction 0 is the buffer above the gel; fraction 1 is the origin. (A) B-16 Sp; (B) E. coli Sp; (C) B-16 Pd hybridized with B-16 Sp; (D) B-16 Pd plus E. coli Sp; (E) E. coli Pd plus E. coli Sp; (F) E. coli Pd plus B-16 Sp.

a polyacrylamide gel (Fig. 2). This was observed with the E. coli strain as reported previously by Cox and Gibson (4) and with the marine pseudomonad.

In another experiment, depleted particles from one source were mixed with the crude soluble ATPase of the other. Again, association occurred between the ATPase and the membrane as evidenced by the appearance of DCCD sensitivity (Table 2) and loss of migrating ability on polyacrylamide gels (Fig. 2). It should be noted that in most cases a certain amount of ATPase activity did not bind and entered the gel. The reason for this is unknown.

This study illustrates the danger of using the classic ATPase assay, ATP hydrolysis, without proper precautions on the effect of other phosphate-hydrolyzing enzymes such as alkaline phosphatase. It also partially characterizes the ATPase in this organism as a DCCD-sensitive enzyme that is most probably attached to the cytoplasmic membrane. The similarity between the enzyme system of E. coli and that of the marine pseudomonad is apparent because both lose sensitivity to DCCD when removed from the cytoplasmic membrane and regain it on reassociation. The bonding that seems to hold the enzymes to the DCCD-sensitive sites on the membrane appears to be similar, being broken in low-ionic-strength aqueous phases. Furthermore, the similarity is strengthened when hybrid ATPase-membrane complexes were prepared from E. coli and marine pseudomonad B-16 components.

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LITERATURE CITED


