Properties and Function of the Proton-Translocating Adenosine Triphosphatase of Clostridium perfringens

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Growth of Clostridium perfringens was inhibited by compounds which dissipate or prevent the formation of electrochemical proton gradients. Membrane vesicles prepared from this organism exhibited Mg²⁺-dependent adenosine triphosphatase (ATPase) activity sensitive to N,N'-dicyclohexylcarbodiimide. Mg²⁺-ATPase activity was optimal at 50°C, but no discrete pH optimum was observed. Adenosine triphosphate-dependent quenching of the fluorescence of the weak base quinacrine by everted membrane vesicles suggested that the Mg²⁺-ATPase is a proton pump capable of generating an electrochemical proton gradient. Adenosine triphosphate-dependent transport of Ca²⁺ by everted vesicles was sensitive to uncouplers and inhibitors of the Mg²⁺-ATPase.

The ability to form and utilize electrochemical ion gradients appears to be a requirement for life under all but the most artificially controlled conditions (6, 9). In most procaryotes proton-translocating electron transport chains form proton motive forces, using various compounds as oxidants and reductants (17). In most of these organisms an enzyme complex known variously as the Mg²⁺-adenosine triphosphatase (ATPase), H⁺-translocating ATPase, or BF₆F₁ utilizes the proton motive force generated by respiration for the formation of ATP (9, 13). In the most widely known case, O₂ is the oxidant, and the process is known as oxidative phosphorylation.

The question has arisen as to the necessity for mechanisms of generation of electrochemical proton gradients in phylogenetically ancient organisms which lack respiratory chains (17). It is likely that the clostridia arose before the evolution of proton-translocating electron transport chains (5, 7). Growth of one species, Clostridium pasteurianum, is inhibited by proton conductors and inhibitors of BF₆F₁ complexes, suggesting that a proton motive force is necessary for growth (11). Transport of sugars is linked to the proton motive force through symport reactions (3). A clostridial ATPase similar to the BF₆F₁ of other organisms has been isolated and purified in several laboratories (4, 10).

Our investigations were designed to answer the following questions: (i) does the related organism Clostridium perfringens have a BF₆F₁, and (ii) if so, is it involved in the generation of an electrochemical proton gradient? If the answer were positive, then it would be expected that growth of C. perfringens would be sensitive to uncouplers and BF₆F₁ inhibitors. We found that when C. perfringens ATCC 3624 was grown anaerobically in a semidefined medium (4), growth was completely prevented by 0.1 mM N,N'-dicyclohexylcarbodiimide, a BF₆F₁ inhibitor, or 5 μM carbonyl cyanide p-trifluoromethoxyphenylhydrazone, an uncoupler, suggesting that electrochemical proton gradients were a part of the energy economy of C. perfringens.

Membrane vesicles could be prepared from lysozyme-treated cells by two methods: lysis with a French press or by sonication. The yield of membranes by the former procedure was proportional to the pressure used over the range of 281 kg/cm² (4,000 lb/in²) to 1,408 kg/cm² (20,000 lb/in²). ATPase activity could be detected in all preparations, but the overall yield decreased with increasing pressure, with maximal yields and specific activity attained at 281 kg/cm². Lysozyme-treated cells sonicated under N₂ for 1 h in a Branson 12 ultrasonic bath at 4°C yielded vesicles with lower specific activity (0.7 to 0.8 U/mg of membrane protein) when compared with vesicles made by French press lysis at 281 kg/cm² (1.0 to 1.3 U/mg of membrane protein). However, the vesicles produced by sonication exhibited a 40% stimulation after Triton X-100 treatment, whereas French press vesicles showed no such crypticity. This suggests that vesicles produced by French press lysis are everted or unsealed, whereas sonic vesicles are sealed and are either a mixture of right-side-out and everted vesicles or have had 60% of the Mg²⁺-ATPase activity translocated to the external surface of the vesicles, as has been observed with vesicles from Escherichia coli (1, 2). ATP-
ase activity required MgCl₂, with an optimum of 10 mM when 5 mM ATP was used. Little activity was observed at 23°C, and the temperature optimum was 47 to 50°C. Activity was constant over a pH range of 5 to 9. ATPase activity was inhibited 80% by 20 μM N,N'-dicyclohexylcarbodiimide and 95% by 50 μM inhibitor.

Everted vesicles from E. coli form electrochemical proton gradients acid and positive interior (1).Weak lipophilic bases distribute across the membrane in response to the formation of the chemical gradient of protons, so that uptake of those weak bases can give a measure of the ΔpH portion of the proton motive force. The aminoacidine quinacrine is one such weak base. Quinacrine is fluorescent, and the fluorescence is quenched upon energization of the membrane. Thus, quenching of quinacrine fluorescence indicates that a ΔpH, acid interior, and, thus, an electrochemical proton gradient have been formed (15).

The sensitivity of the Mg²⁺-ATPase to N,N'-dicyclohexylcarbodiimide suggested a proton-translocating function. Addition of ATP to sonic vesicles from C. perfringens produced quenching of quinacrine fluorescence (Fig. 1). Although French press vesicles were not cryptic and had higher specific activity than sonic vesicles, they did not exhibit energy-linked quenching of quinacrine fluorescence. Sonic vesicles did not quench quinacrine fluorescence when pretreated with N,N'-dicyclohexylcarbodiimide or when ATP was replaced with ADP (Fig. 1). Activity was observed at 40°C but not at 23°C, consistent with the high temperature optimum of the Mg²⁺-ATPase. These results suggest that the Mg²⁺-ATPase of C. perfringens is a proton-translocating BF₂F₁, establishing a proton motive force through hydrolysis of ATP.

Since C. perfringens lacks oxidative phosphorylation, what is the function of a proton motive force? The most likely one is to supply energy for transport. Calcium is actively extruded by most procaryotes (16), with energy supplied by the proton motive force (18) or by ATP directly (8). Uptake by everted vesicles is considered equivalent to extrusion by cells. With conditions similar to those used for E. coli (14), we observed uptake of ⁴⁰Ca²⁺ into sonic vesicles (Table 1). Again, no activity was observed below 40°C. ATP was required; ADP could not substitute. Either N,N'-dicyclohexylcarbodiimide or carbonyl cyanide p-trifluoromethoxyphenylhydrazone inhibited (Table 1), demonstrating that a proton motive force rather than ATP directly was required.

In conclusion, it now appears that the F₂F₁ complex is ubiquitous among procaryotes, from aerobes to facultative anaerobes to obligate an-

![Figure 1](http://jb.asm.org/article-pdf/84/1/746/3783670/0095-1682-84-1-746.pdf)

**FIG. 1.** ATP-dependent quenching of quinacrine fluorescence by sonic vesicles. Quinacrine fluorescence was assayed at 42°C as described previously (12). Additions were made at the times indicated by the arrows. (Curve a) 0.1 mM N,N'-dicyclohexylcarbodiimide (DCCD) added before addition of 6 mM ATP; (curve b) 6 mM ADP; (curve c) 6 mM ATP.

aerobes. One function in most organisms is the formation of ATP coupled to an electron transport system (9, 13), whether NADH/O₂ coupled, as in aerobes, fumarate/nitrate coupled, as in
TABLE 1. Effect of N,N’-dicyclohexylcarbodiimide and carbonyl cyanide p-trifluoromethoxyphenylhydrazone on uptake of \(^{40}\text{Ca}^{2+}\) into membrane vesicles

<table>
<thead>
<tr>
<th>Energy source</th>
<th>Addition</th>
<th>(^{40}\text{Ca}^{2+}) uptake* (nmol/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>7.6</td>
</tr>
<tr>
<td>5 mM ATP</td>
<td>None</td>
<td>33.0</td>
</tr>
<tr>
<td>5 mM ATP</td>
<td>0.1 mM N,N’-dicyclohexylcarbodiimide</td>
<td>8.8</td>
</tr>
<tr>
<td>5 mM ATP</td>
<td>5 \mu M carbonyl cyanide p-trifluoromethoxyphenylhydrazone</td>
<td>9.6</td>
</tr>
</tbody>
</table>

* Assays were performed as described previously (14) at 42°C for 20 min.

facultative anaerobes, or H\(_2\)/CO\(_2\) coupled, as in methanogens (17). The existence of a BF\(_{1}\) complex in organisms which probably arose before evolution of proton-translocating electron transport chains suggests that the original function of the complex was something other than ATP formation. The obligate anaerobe C. perfringens requires a proton motive force for growth (11) and for transport (3). The organism has a BF\(_{1}\)F\(_{1}\) similar to that of other procaryotes (4, 10), leading naturally to the assumption that this complex is the generator of electrochemical energy in that organism. We have demonstrated that a similar complex exists in C. perfringens and that the BF\(_{1}\)F\(_{1}\) of C. perfringens is capable of generating proton gradients through hydrolysis of ATP. Finally, we have shown that transport of calcium can be coupled to that source of energy. These results support the concept that a proton circulation composed of an H\(^+\)-translocating ATPase and of secondary active transport systems is essential to the existence of the obligate anaerobes which lack respiratory chains.

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