Permeabilization of Isolated Heterocysts of *Anabaena* sp. Strain 7120 with Detergent

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Heterocysts isolated from *Anabaena* sp. strain 7120 with lysozyme plus sonication were permeabilized with the cationic detergent cetyltrimethylammonium bromide, and they then exhibited comparable acetylene reduction activity in the light and dark with an ATP-regenerating system plus dithionite. The detergent diminished the effect of H$_2$ in enhancing acetylene reduction.

Biological N$_2$ fixation has an absolute requirement for ATP and reductant, and the supply of either may regulate nitrogenase activity in vivo. The heterocystous cyanobacterium *Anabaena* provides a convenient system for study of the complex processes related to N$_2$ fixation. In aerobically grown filaments, the nitrogenase components are compartmentalized within heterocysts (7, 8, 10, 12). These cells, which are not susceptible to digestion by lysozyme, can be isolated easily, and their N$_2$ fixation can be studied in situ. However, isolated heterocysts differ in their degree of intactness and thus in their permeability to exogenous compounds. To study the effects of exogenous nucleotides on the nitrogenase activity of isolated heterocysts, it is necessary to assure permeation of these compounds into the heterocysts. Toluene has been used to permeabilize *Anabaena* sp. CA (5), but nitrogenase activity has been lost during anaerobic treatment with toluene. The cationic detergent cetyltrimethylammonium bromide (CTAB) has been found to be effective in maintaining the adenylation state of glutamine synthetase during its extraction from certain bacteria (3). We have found that treatment of heterocysts with CTAB (0.0125%) permeabilizes them to exogenous nucleotides but does not destroy their ability to reduce acetylene.

*Anabaena* sp. strain 7120 (ATCC 27893, *Nostoc muscorum*) was grown and heterocysts were isolated as described previously (11). Isolated heterocysts were treated with 0.0125% CTAB or other detergents for 10 min on ice. Then they were washed twice by centrifugation and suspended in 40 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid)—1 mM MgCl$_2$, pH 7.4

Hydrogen uptake in the dark was measured with an H$_2$ electrode (13) at 30°C; the initial H$_2$ concentration was 38 μM. Acetylene reduction assays for measuring nitrogenase activity (6) were run for 20 min at 30°C in an illuminated water bath with shaker (850 μEinstein per m$^2$·s). Gas samples were analyzed with a Carle gas chromatograph with flame ionization detector.

Endogenous phosphorylation was measured by following esterification with $^{32}$P in 0.1-ml reaction mixtures containing heterocysts (ca. 30 μg of chlorophyll), 20 μM Tricine, pH 9.0, 5 mM MgCl$_2$, 10 mM KCl, 2 mM ADP, 2 mM Pi, 30 mM glucose, and 5 μg of hexokinase. Reactions were run for 20 min under air or argon with and without illumination (850 μEinstein per m$^2$·s). Reactions were terminated with 2 ml of 1 M perchloric acid—5% ammonium molybdate (4:1); esterified $^{32}$P was extracted by the procedure of Avron and Schreiber (2).

Chlorophyll was determined in 80% acetone extracts by the method of Vernon (14).

Isolated heterocysts have effective access to the medium only at the narrow opening exposed at their polar regions when the heterocysts are broken away from adjacent vegetative cells. Exogenous compounds must enter and endogenous compounds must leak out through these regions. We found that heterocysts isolated by our procedure had nitrogenase activity that could be enhanced in the light by H$_2$ dithionite, or an ATP-regenerating system plus dithionite. In the dark, neither H$_2$ nor dithionite supported acetylene reduction. If ATP and reductant are present and penetrate the heterocysts, activity in the dark should be comparable to that in the light; this was not observed (Table 1, column 1). After treatment with CTAB, however, an ATP-regenerating system plus dithionite supported acetylene reduction by washed heterocysts comparably in the light and dark; the response was greater than for untreated heterocysts (Table 1, column 2). After CTAB treatment, responses in
the light to H₂ and dithionite were much lower than those observed for control heterocysts. Among other detergents tested, Triton X-100, a nonionic detergent, apparently increased heterocyst permeability to ATP, whereas the anionic detergent sodium dodecyl sulfate did not. Only CTAB and dodecyltrimethylammonium bromide (DTAB) diminished the H₂-enhanced activity.

Hydrogen probably supports activity in isolated heterocysts via an uptake hydrogenase (4,9). This hydrogenase catalyzes the oxyhydrogen reaction and can recycle energy otherwise dissipated as H₂ produced by nitrogenase. Reducant or ATP may be reclaimed in this process. Treatment with CTAB adversely affected H₂-supported nitrogenase activity but not by inhibition or inactivation of the uptake hydrogenase. H₂ uptake was not light dependent, and similar specific activities (3.82 versus 4.21 nmol per (min · μg of chlorophyll) were obtained in CTAB-treated and control heterocysts. The cationic detergent, DTAB, decreased H₂-enhanced acetylene reduction much like CTAB, but the nonionic detergent Triton X-100 or the anionic detergent sodium dodecyl sulfate did not affect this response to H₂. Thus, this effect is not general for detergents but seems to be specific for cationic detergents.

Endogenous phosphorylation of CTAB- and non-CTAB-treated heterocysts was measured by incorporation of ³²P from phosphate (Table 2). Phosphorylation was measured in an air or argon atmosphere with or without 50% H₂ in the light and dark. Levels of phosphorylation were comparable to those reported for endogenous phosphorylation by Almon and Bohme (1). Non-treated heterocysts supported phosphorylation plus substrate-level phosphorylation under argon in the light, and they supported oxidative phosphorylation plus substrate level phosphorylation under air in the dark. CTAB treatment reduced the endogenous phosphorylation in isolated heterocysts and eliminated the H₂-enhanced oxidative phosphorylation. This implies that although CTAB treatment does not affect the hydrogenase it does disrupt electron transport or uncouple oxidative phosphorylation and photophosphorylation.

Our search for an agent to permeabilize isolated heterocysts revealed that CTAB increases permeability to exogenous nucleotides but also adversely affects H₂-supported phosphorylation in heterocysts. This effect may prove useful in the study of the metabolism of H₂ and the path of electron transfer in the oxyhydrogen reaction of other organisms.

TABLE 2. Effects of CTAB-treatment on endogenous phosphorylation by isolated heterocysts

<table>
<thead>
<tr>
<th>Atmosphere</th>
<th>Control</th>
<th>CTAB treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Light</td>
<td>Dark</td>
</tr>
<tr>
<td>Argon</td>
<td>25.8 ± 2.0</td>
<td>7.5 ± 1.2</td>
</tr>
<tr>
<td>50% Argon + 50% H₂</td>
<td>19.5 ± 3.5</td>
<td>15.0 ± 0.7</td>
</tr>
<tr>
<td>Air</td>
<td>68.9 ± 2.2</td>
<td>56.6 ± 4.5</td>
</tr>
<tr>
<td>Air + 50% H₂</td>
<td>80.5 ± 1.9</td>
<td>64.6 ± 7.5</td>
</tr>
</tbody>
</table>

*Phosphorylation values are expressed as μmol of P₃ esterified h⁻¹ mg of chlorophyll⁻¹.
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LITERATURE CITED