pH Changes in the Cytoplasm of the Blue-Green Alga *Anacystis nidulans* Caused by Light-Dependent Proton Flux into the Thylakoid Space

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**ABSTRACT**

The pH in the cytoplasmic and thylakoid spaces of the blue-green alga, *Anacystis nidulans*, has been determined in the light and in the dark by uptake of 5,5-dimethylxazolidine-2,4-dione and methylamine into the sucrose-impermeable 3H-H2O space, as measured by silicon layer filtering centrifugation. Illumination causes an alkalization in the cytoplasm which is accompanied by an acidification in the thylakoid space, reflecting light-dependent proton transport across the thylakoid membrane. Under light conditions, a pH gradient of approximately 2.8 between the cytoplasmic and thylakoid spaces has been measured that can be abolished almost completely by addition of the uncoupler, 3-chlorocarbonyl cyanide phenylhydrazone. The pH in the cytoplasm is independent of the pH in the medium.

Heldt et al. (3) have shown with intact spinach chloroplasts that illumination causes an alkalization of the stroma space which is accompanied by acidification of the thylakoid space, due to the light-dependent proton flux across the thylakoid membrane. Because of the importance of this alkalization in the stroma for the regulation of CO2 fixation in chloroplasts (6), it was interesting to see if this phenomenon also occurs in a different biological system possessing a membrane with photosynthetic electron transport, thus representing a general feature of photosynthetic control. We have chosen the blue-green alga, *Anacystis nidulans*, which is known to have a type of thylakoid membrane capable of photosynthetic electron transport. Hence, the whole algal cell can be compared from a structural point of view with the intact chloroplast. Information concerning such a light-dependent pH change across the thylakoid membrane would also provide insight into the energy state of the algal cell due to photosynthesis. The measurement of the phosphorylation potential during photosynthetic activity has been found to be complicated, since it is difficult to stop metabolism quickly and to determine the concentration of free inorganic phosphate in the cell. Determination of the pH in the cytoplasmic and thylakoid spaces by the method used with chloroplasts would avoid this experimental difficulty.

**MATERIALS AND METHODS**

*Anacystis nidulans* Dr. strain 1402-1 (Algal Culture Collection, Göttingen) was cultivated in the medium of Kratz and Myers (5), at a light intensity of 15,000 lux and a temperature of 38 C. For measurement of the pH in the cytoplasmic space and in the thylakoid space, the algae were incubated in triplicate in a medium containing 50 mM HEPES-NaOH (pH 7.3), 1 mM DMO, and 70 μM methylamine containing either 14C-DMO (New England, 0.1-1 Ci/mol) or 14C-methylamine (Amersham, 20 to 40 Ci/mol).

3H-H2O (Amersham, 0.4 Ci/mol) and 14C-sucrose (New England, 0.1 Ci/mol) were used to determine the sucrose-impermeable 3H-H2O space (2). Filtering centrifugation was performed as described by Klingenberg and Pfaff (4). Illumination was provided by a tungsten-halogen light source, and continued during centrifugation. The light intensity was 15,000 lux. All experiments were carried out at 20°C.

The principle of the method of pH measurement, utilizing the distribution of DMO and methylamine inside and outside the cell, has been described elsewhere (3). The intracellular space was defined as the sucrose-impermeable 3H-H2O space (2). The thylakoid space was assumed to be 7% of the intracellular space; this value is based on a planimetric estimation by Allen (1). There are two factors that complicate the pH measurement with *Anacystis*. First, it is possible that methylamine could undergo metabolic changes. For this reason, the substance was not incubated longer than 2 min. During this period of time, any errors due to metabolic conversion were within the standard deviation of the method. Any change of the pH or redistribution of methylamine between inside and outside the cell caused by light-dark alterations occurs within 40 sec. Secondly, the accuracy of the absolute pH values may be affected to a certain extent by the influence of the several layers of the cell wall on the sucrose space.

A linear relationship between the 5,5-dimethylxazolidine-2,4-dione anion concentration in the medium and in the large sucrose-impermeable 3H-H2O space was noted over a large concentration range (0.1-6.0 mM 5,5-dimethylxazolidine-2,4-dione); that is, the results of the pH measurement in the cytoplasm are independent of the DMO concentration. With methylamine, such a linearity exists only when the methylamine concentration is below 70 μM, almost certainly as a result of the uncoupling effect of methylamine at higher concentrations. The concentration of methylamine employed for pH measurement in the thylakoid space was, therefore, 70 μM.

**RESULTS AND DISCUSSION**

Values for the pH in the cytoplasmic and in the thylakoid space in the light and in the dark are given in Table 1. As with

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1 Abbreviations: DMO: 5,5-dimethylxazolidine-2,4-dione; CCCP, carbonyl cyanide m-chlorophenylhydrazine.
Table 1. *pH in the Cytoplasmic and Thylakoid Spaces in the Light and in the Dark*

<table>
<thead>
<tr>
<th></th>
<th>pH Cytoplasm</th>
<th>pH Thylakoid</th>
<th>Δ pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>7.5</td>
<td>4.6</td>
<td>2.9</td>
</tr>
<tr>
<td>Dark</td>
<td>6.9</td>
<td>5.2</td>
<td>1.7</td>
</tr>
</tbody>
</table>

![Fig. 1. Effect of CCCP on the light-dependent pH changes in the cytoplasmic and thylakoid spaces.](image1)

![Fig. 2. pH changes in the cytoplasm and thylakoid spaces caused by dark-light and light-dark alterations after the addition of methylamine and DMO.](image2)

![Fig. 3. Relationship between pH in the cytoplasm and pH in the medium, in the light, and in the dark (50 µg Chl/ml).](image3)

dium, it was interesting to see whether this is also the case with the blue-green alga. It is clear from Figure 3 that the pH in the cytoplasm is completely independent of the pH in the medium, indicating that the membrane in the cytoplasm is impermeable to protons under these experimental conditions.

The pH values of Table I are typical for algae grown in the medium of Kratz and Myers (5), although absolute values for the pH vary considerably with different algal preparations. Furthermore, it can be shown that the pH also depends on a number of factors which influence pigmentation concentration in the thylakoid membrane. Algae cultivated under different nutrient conditions show not only a considerable variation in the pH gradient in the light, but also the gradients observed in the dark vary to a certain extent (unpublished observations). The pH gradients presented here are maximum values which have been obtained when the algae were grown under optimal nutrient conditions.

The fact that both chloroplasts and a blue-green alga, which exist under totally different environmental conditions, behave in a similar manner in regard to the pH change across the thylakoid membrane suggests a close relationship between these two structures from an energetic point of view. Studies with isolated chloroplasts have shown that light-induced alkalinization of the stroma may be a major factor in the light control of CO₂ fixation (6). We found this alkalinization also in the blue-green algae. This indicates that the control of CO₂ fixation by pH may be a general mechanism for the regulation of photosynthesis.

**LITERATURE CITED**