Photosynthetic Properties of Permeaplasts of *Anacystis*

BAILEY WARD and JACK MYERS

Departments of Botany and Zoology, The University of Texas, Austin, Texas 78712

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ABSTRACT

A treatment procedure using lysozyme and ethylenediaminetetraacetic acid gave intact but permeable cells (permeaplasts) of *Anacystis nidulans*. Rates of electron transport from water to carbon dioxide, ferricyanide, 2,6-dichlorophenol indophenol, benzoquinone, and methyl viologen, and from reduced indophenol to methyl viologen were measured as a function of treatment time. Rates of oxygen evolution in complete photosynthesis and electron flow from water to methyl viologen showed rapid and parallel decline with treatment time. Electron flow from water to ferricyanide and from reduced indophenol to methyl viologen increased during the first half hour of treatment (phase 1) to 60 to 80% of the original photosynthetic rate. Longer treatment (phase 2) resulted in decreased rate of ferricyanide reduction but not in rate of methyl viologen reduction from indophenol. Electron flow from water to quinone was two to three times higher than for complete photosynthesis in intact cells. It remained high during phase 1 and declined during phase 2. Phase 1 permeaplasts apparently retain high activity for photosystems 1 and 2 photoreactions.

Various means of disruption have been used to obtain particulate preparations from blue-green algae with photochemical activities measured in terms of photophosphorylation or electron transport to selected acceptors. Less attention has been given to development of milder treatments. It would be of obvious advantage to have a preparation of photosynthetic procaryotic cells, functionally analogous to chloroplasts of eucaryotes: a high fraction of original activity coupled with accessibility to additives. Attempts in this direction have been made by using lyophilization (6), lysozyme treatment (2, 4, 8, 15), and a combination of the two (5).

Our attempts to obtain reproducibly a permeable but intact cell preparation met with little success until we tried the combination of lysozyme and EDTA found useful for solubilizing the cell wall of *Pseudomonas aeruginosa* (3). Although we were aware of possible internal damage which could be caused by EDTA, we have developed a treatment procedure which reproducibly gives a preparation of intact but permeable cells (permeaplasts) with isolated photosystems 1 and 2 of high ability, although with lowered rates of complete photosynthesis.

MATERIALS AND METHODS

Preliminary trials with several blue-green algae led to selection of *Anacystis nidulans* Richter, Kratz and Allen strain.

This alga is less sensitive to lysozyme than others examined and is easily manipulated. Cultures were grown in medium C (7), modified by doubled concentrations of iron and microelements, in a continuous culture chamber (11) at 30 C, and aerated with 1% CO₂ in air. At 100 ft-c from tungsten lamps and a cell concentration of 2.5 µl cells/ml, the specific growth rate was about 0.9/day.

A standard permeaplast preparation was obtained by the following procedure. A harvested cell suspension was centrifuged at 12,000g for 5 to 10 min at 0 C, and the cells were resuspended in 50 mm TES buffer at pH 7.3 to give 7 to 10 µl cells/ml. Lysozyme ( Worthington Biochemicals, 2X recrystalzied) was added to give 1.5 to 2.0 mg/µl cells, and Na₂EDTA was added to give 1.0 mm. Cell suspensions were incubated in 15-ml tubes, about 5 ml/tube at 35 C in room light with occasional stirring by a Vortex mixer. After desired time of treatment, aliquots of suspension were diluted in several volumes of cold TES buffer, centrifuged for 5 to 10 min at 12,000g at 0 C, washed in TES buffer, and resuspended in room temperature TES to give cell concentrations of 0.5 to 2.0 µl/ml for assay. Most preparations gave relatively stable activities for about 1 hr when maintained at room temperature after the initial centrifugation.

Oxygen exchange was measured by a Beckman oxygen macroelectrode in a cuvette of about 1.5 ml (12) or by a YSI 5331 electrode in a 1.6-ml cuvette at 25 C (17). Rate-saturating illumination was provided by projection systems via interference filters (Baird Atomic) at 600 or 620 nm, half-band widths 112 and 150 nm. Cell suspensions were equilibrated with 1% oxygen and 2% carbon dioxide in nitrogen before measurement. Photoreduction of methyl viologen was measured by oxygen uptake in the presence of air, catalase, and ethanol with an assumed stoichiometry of four electrons/O₂.

Measurements of absorption changes of DPIP and ferricyanide were made by a single beam spectrophotometer using 589 nm and ε₅₈₉ = 21 for DPIP and 420 nm and ε₄₂₀ = 0.9 for ferricyanide. A Beckman DU spectrophotometer was provided with a Syvania 6.6A tungsten-halogen lamp operated from a storage battery and with a Gilson Model 220 photometer. A rate-saturating actinic beam at right angle to the measuring beam was provided by a projection system through a 1.0-cm water cell, an interference filter (Bausch and Lomb 630 nm, half-band width 20 nm), and Corning 2404 and 2412 filters. Appropriate filters in front of the photomultiplier minimized stray light. Absorbance changes were measured at 0.1 A full scale. For experiments with semicarbazide and diphenylcarbazide electron donors, a concentrated permeaplast preparation was held at 4 C prior to assay and diluted in room temperature TES for assay; rate of DPIP or ferricyanide photoreduction was then measured before and after addition of the donor.

Chlorophyll a was determined by a Cary Model 14 spectro-

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2 Present address: Department of Biology, University of Mississippi, University, Miss. 38677.

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*Abbreviation: DPIP: 2,6-dichlorophenol indophenol.*
RESULTS

Permeaplast Characteristics. With time of treatment the cells became more transparent and formed irregular, semispherical bodies which tended to aggregate. About 10% of the cells showed no changes observable under phase contrast microscopy. Aggregates could be broken by vibration of a tube on a Vortex mixer. A gradual loss of phycocyanin, about 30% in 2 hr, accompanied observable microscopic changes. At higher concentrations of EDTA (0.1 M) plus lysozyme at standard concentration the cells became uniformly spherical after 2 hr, lysed on dilution, and allowed recovery of a membrane fraction nearly free of phycocyanin. However, the present report concerns only permeaplasts, intact cells modified in morphology and behavior by the mild, standard treatment regime.

Photoreactivities of Permeaplasts. With time of treatment there was a regular and continuous decline in rate of oxygen evolution in the absence of added electron acceptors (Fig. 1). Treatments with 1 mM EDTA or lysozyme alone gave only about 20% decrease in photosynthetic rate; only the lysozyme-treated cells would slowly reduce ferricyanide. Untreated cells did not photoreduce ferricyanide nor was their rate of oxygen evolution affected by addition of ferricyanide. However, relatively short standard treatment gave increasing rates of ferricyanide photoreduction which reached 80% of control and could be observed also in terms of oxygen evolution (Fig. 1). Extended treatment revealed a biphasic characteristic observed in ferricyanide photoreduction: initial high rates remained constant from about 20 to 60 min and then progressively decreased (Fig. 2). Oxygen evolution measured with DPIP as oxidant also showed the biphasic phenomenon. However, maximum rates of DPIP photoreduction were consistently lower than those obtained with ferricyanide, even when the pH was adjusted to yield optimum rates (pH 6–7) and corrections made for the molar absorption coefficient of DPIP (1).

With methyl viologen as electron acceptor (water serving as reductant) rates of electron transport declined steadily with treatment time, although they remained consistently higher than rates of electron transport to CO2. Isolated photosystem 1 rates (DPIPH2 plus DCMU), which were low, but measurable in intact cells, increased to relatively high values, sometimes reaching 70% of control and remaining constant (Fig. 2). Representative Hill activities are presented in Table I.

Our attempts to restore electron transport to CO2 or to methyl viologen by addition of mono- or divalent cations or by addition of concentrators from supernatants were unsuccessful. We had limited success in partly restoring ferricyanide and

Photometer on an extract in 80% (v/v) acetone using a specific absorption coefficient of 82.0 at 663 nm (10).

Table I. Representative Electron Transport Rates

RATES are compiled from measurements of oxygen exchange with added electron acceptors. Permeaplasts were prepared according to standard treatment. Reaction conditions are as described for Figure 2.

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>Electron Transport Rates after Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td></td>
<td>μeq/mg chl-μhr</td>
</tr>
<tr>
<td>CO₂</td>
<td>800–900</td>
</tr>
<tr>
<td>DPIP</td>
<td>0²</td>
</tr>
<tr>
<td>Ferricyanide</td>
<td>0²</td>
</tr>
<tr>
<td>Methyl viologen</td>
<td>700–800</td>
</tr>
<tr>
<td>Methyl viologen, DCMU, DPIPH₂</td>
<td>50–150</td>
</tr>
</tbody>
</table>

² No appreciable changes in rate of oxygen evolution occurred upon addition of the oxidant.
DPIP photoreduction by use of artificial electron donors (16), and then only in phase 2 permeaplasts after extended treatment. When rate of ferricyanide photoreduction had decreased to 140 μeq/mg chl-hr, it was increased 160% by addition of semicarbazide. When rate of DPIP photoreduction had decreased to 70 to 80 μeq/mg chl-hr, it could be increased 30 to 200% by the addition of 1,5-diphenylcarbazide. Rates of ferricyanide photoreduction in phase 1 permeaplasts were increased only 10 to 20% by phosphorylating conditions (5 mM K, HPO₄, 3 mM MgCl₂, 3 mM ADP) or addition of an uncoupler (5 mM methyl amine).

The Special Case of Benzoquinone. Photoreduction of benzoquinone could be readily observed in both treated and untreated cells by following the reduction of ferricyanide (6, 13). Figure 3A shows rates as a function of quinone concentration. The remarkable feature is that at optimum quinone concentration electron transport in untreated cells and in phase 1 permeaplasts is two to three times that observed during normal photosynthesis. The rates had dropped to 50% of the rate observed during normal photosynthesis. Equivalent to 2 to 3 times the rate observed during normal photosynthesis. The rates had dropped to 50% of the rate observed during normal photosynthesis.

DISCUSSION

The immediate and dramatic effect of lysozyme-EDTA treatment is a rapid rise in rate of ferricyanide photoreduction from zero to about 80% of the electron throughput rate to carbon dioxide observed in untreated cells. We attribute the increase in rate of ferricyanide reduction to increase in permeability of the cell wall/membrane barriers. The term permeaplast provides a functional rather than a morphological description, although the cells appear more closely related to "spheroplasts" than to "protoplasts," since electron microscopy has shown that at least one cell wall layer remains (9). In the context of the Z-scheme, we interpret the early rapid decrease in rate of electron transport to methyl viologen (and to CO₂) mainly as a loss or inactivation of factor(s) operating prior to photosystem 1. During the same early period, the isolated photosystem 1 reaction (DPIP/H₂ to methyl viologen) and the photosystem 2 reduction of ferricyanide were increasing. Hence we ascribe the early effects of treatment in phase 1 permeaplasts to impairment of electron transport between the photoauts. We were disappointed by our inability to restore this loss by additives. With further treatment, phase 2 permeaplasts showed decreasing rates of ferricyanide, DPIP, and quinone reduction as might be expected of impairment of system 2 activity. We were able to restore partially rates of ferricyanide and DPIP reduction by addition of the artificial donors semicarbazide or diphenylcarbazide, but only when the rates had dropped to the low levels of phase 2 permeaplasts. We interpret these results to mean that prolonged treatment resulted in some impairment to the photosystem 2 apparatus.

We consider benzoquinone a special case. We confirm an earlier discovery by Van Baalen (14) that in intact cells of Anacystis the rate of oxygen evolution with added quinone may be two to three times the rate observed during normal photosynthesis. Equivalent rates are also observable in terms of ferricyanide reduction with added quinone and are only slightly reduced in phase 1 permeaplasts. We note the proposition of Saha et al. (13) that the quinones and quinonemides belong to a special class of electron acceptors (class III) usually with high lipid solubility, anomalously high rates of electron transport, and with lowered phosphorylating efficiency. Since in our case we compare direct rates of electron transport in intact cells with and without quinone, we support their conclusion that quinone may accept electrons prior to the rate-limiting step in photosynthesis.

We presume that the increased accessibility to oxidants extends also to other possible additives not included in our initial exploration. If this proves to be true then the mildly treated permeaplast preparation of Anacystis offers potential advantages such as have been developed for chloroplasts of eucaryotic plants. At the present stage of development the phase 1 permeaplast preparation provides high residual activity for the photosystems 1 and 2 photoreactions.

LITERATURE CITED