Action Spectra for Photosystems I and II in Formaldehyde Fixed Algae

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Abstract. Action spectra were obtained for photosystems I and II in chemically fixed algal cells and for photosystem I in unfixed lysozyme treated cells. Untreated algal cells yielded neither of the 2 light reactions with the reaction mixtures used. The action spectra for photosystem I in the blue-green alga Anacystis nidulans and red alga Porphyridium cruentum follow the absorption spectrum of chlorophyll a with a small peak in the region of the accessory pigments. In the green alga Chlorella pyrenoidosa the photosystem I action spectrum follows the absorption spectrum of chlorophyll a. Photosystem II action spectra in A. nidulans and P. cruentum follow the absorption spectra of the accessory pigments while that in C. pyrenoidosa is shifted slightly toward the blue spectral region. These results provide additional evidence that formaldehyde fixed cells are valid models for studying the light reactions of photosynthesis.

Photosynthetic action spectra were determined for brown, green, and red algae by Haxo and Blinks (6), using polarographic oxygen determinations. They found that the action spectra for photosynthesis in brown and green algae agreed with the absorption spectra for these algae, but that in red algae the action spectrum for photosynthesis followed primarily the absorption of light by the accessory pigment, phycocyanin, with chlorophyll appearing largely inactive. Their work, with that of Emerson on the red drop, posed the problem of inactive chlorophyll in plants; a problem finally resolved by the recognition of 2 photochemical systems in photosynthetic electron transport. Kok and Hoch (10) reported the photosynthetic action spectrum of the blue-green alga Anacystis nidulans which showed that light absorbed by chlorophyll a alone was much less efficiently used than light absorbed by both phycocyanin and chlorophyll. The oxidation of P-700 in Anacystis nidulans was driven primarily by chlorophyll a whereas its reduction was driven primarily by the accessory pigment, an observation which could be most easily explained by the existence of 2 light reactions. Duysens et al. (4) from action spectra for cytochrome oxidation and reduction in the red alga Porphyridium cruentum proposed the existence of 2 photosystems, 1 peaking at 560 nm and the other at 680 nm. These data also suggested that the 2 photochemical systems were related in a sequential manner. Though the 2 light reactions of photosynthesis can be studied in green cells or fragments from green cells that do not contain accessory pigments (7), they are most easily differentiated in the red and blue-green algae where the 2 photochemical reactions are spectrally widely separated. The general approach in studying the 2 photochemical systems in such organisms has been via cell fractionation studies, such as those carried out by Susor and Krogman (16), and Biggins (1,2). Susor and Krogman studied cell fragments from the blue-green alga Anabena variabilis. Their results showed that only at limiting light intensities did phycocyanin absorbance decrease the Hill reaction. Biggins treated Phormidium luridum with lysozyme and obtained photosynthetically active protoplasts. Further rupture caused loss of accessory pigment from the photosynthetic apparatus.

The photosynthetic organisms with accessory pigments, while spectrally suited for studying the 2 light reactions of photosynthesis, remain impermeable to dyes and other components of photosystem I and photosystem II reaction mixtures unless the cells are broken. However, destruction of the permeability barrier to reaction mixture components leads to dissociation of the accessory pigment from the rest of the quantum conversion apparatus. This paper deals with a new approach for studying photosystems I and II in cells with accessory pigments. We have fixed algal cells in formaldehyde with some modifications from the work of Hallier and Park (5) and report the action spectra obtained from these fixed cells. This rapid method for preparing highly active material for light reaction studies lends further support to the validity of formaldehyde fixed cells as models for studying electron transport in photosynthesis.
Materials and Methods

Algae. *Anacystis nidulans* (Richt.) Drouet and Daily cells were grown on Kratz and Myers' Medium C (11) in 1 liter flasks with continuous shaking. The flasks were flushed with 4% CO₂ in air and illuminated with cool white fluorescent tubes. The temperature was maintained between 33° to 35°.

*Chlorella pyrenoidosa* Chick cells were grown on a modified Myers' Medium (3) at 20°. These cultures were also shaken and flushed with 4% CO₂ in air.

*Porphyridium cruentum* (Agardh) Nageli cells were grown on a modified Jones medium (8) at 21°. These cultures were flushed with 4% CO₂ in air and illuminated with cool white fluorescent tubes.

Preparation of Formaldehyde. Formaldehyde solutions were prepared by heating paraformaldehyde at 75° for 5 min in 0.03 M phosphate buffer (pH 7.4). This solution was filtered and adjusted to pH 7.4. The formaldehyde was made up freshly for each experiment.

Fixation of Cells. *Anacystis nidulans* were fixed in 1.5% formaldehyde (w/v) in 0.03 M phosphate buffer (pH 7.4) for 4 min when studying photosystem II. The cells were preilluminated for 1 hr, fixed and examined for photosystem II activity using a mannitol-tricine buffer (pH 7.4). For photosystem I activity *A. nidulans* cells were prepared according to Biggins (1) with modification. Cells were washed in mannitol-phosphate buffer (0.01 M mannitol, 0.03 M potassium phosphate at pH 6.8) and resuspended in the buffer containing 0.5% lysozyme (w/v) which was obtained from Worthington Biochemical Corporation, Freehold, New Jersey. Incubation was done at 35° for 16 hr. The yield of protoplasts was about 80%. The cells were then cooled, centrifuged and the enzyme washed out with mannitol-phosphate buffer. Photosystem I activity was measured after resuspension of the protoplasts in mannitol-tricine buffer. *Chlorella pyrenoidosa* and *Porphyridium cruentum* cells fixed in 1.5% (w/v) formaldehyde for 10 min also yielded both photosystem I and photosystem II activity when resuspended in mannitol-phosphate buffer (pH 7.4). Fixed material which had been stored for 3 days gave photochemical activity comparable to the first day of fixation.

Biochemicals. Methyl viologen was provided by California Chemical Company, Ortho Division, Richmond, California; 2,6 dichlorophenol indophenol (DCPIP) was obtained from Sigma Chemical Company, St. Louis, Missouri and 3-(3,4-dichlorophenyl)-1,1-dimethyleurea (DCMU) was provided by DuPont Chemical Company, Wilmington, Delaware.

Reaction Mixtures. Photosystem I activity was measured under anaerobic conditions in a standard reaction mixture containing in μmoles/ml: ascorbate, 5; methyl viologen, 5; DCMU, 0.1; DCPIP, 0.05; mannitol, 250; phosphate, 30 or tricine, 50. Photosystem I was measured under anaerobic conditions since methyl viologen in the reduced state is readily oxidized. Nitrogen was bubbled through the sealed reaction mixture for 5 min and then it was illuminated to eliminate the remaining free oxygen prior to determinations. The reaction mixture for photosystem II contained in μmoles/ml: methylamine, 100; DCPIP, 0.05; mannitol, 250; phosphate, 30 or tricine, 50. The chlorophyll concentrations used in photosystems I and II reaction mixtures were 11 and 8 μg/ml of reaction mixture, respectively.

Spectrophotometry. Experiments were carried out using a Cary Model 14 spectrophotometer modified so that the absorption of the dyes could be monitored continuously while the sample was being irradiated from the side as described by Sauer and Biggins (14). Concentration of DCPIP was monitored a 580 nm with a 4-96 Corning filter in front of the photomultiplier tube and methyl viologen concentration was monitored at 395 nm.

Cuvettes had 4 clear sides and a rectangular internal cross section of 3 mm × 10 mm. The monochromatic measuring beam passed through the 10 mm path length and the actinic light traversed the 3 mm light path in the reaction mixture (15). Corrections were made so that equal incident quanta were given at each wavelength, in the intensity range in which rate was proportional to absorption. The maximum rates which fell in this proportional range were for A) photosystem II in μmole dye/mg chloro-
Results and Discussion

Action spectra for both photosystems were determined for *Anacystis nidulans*, *Chlorella pyrenoidosa*, and *Porphyridium cruentum*. All assays were run on formaldehyde fixed cells with the exception that the photosystem I activity of *A. nidulans* was studied in lysozyme treated cells. The action spectra for photosystems I and II as compared with the absorption spectrum of *A. nidulans* are shown in Fig. 1. The action spectrum for photosystem I, in which cells were treated with lysozyme follows the absorption spectrum for chlorophyll a with a slight peak occurring in the region of phycocyanin absorption. The action spectrum for photosystem II, in which cells were fixed in formaldehyde, agrees well with the absorption spectrum of phycocyanin and has a small peak in the chlorophyll a region. Thus, photosystem II is driven primarily by phycocyanin in the fixed cells. Photosystem I is driven primarily by chlorophyll a with a small contribution from phycocyanin. These data are consistent with the photosynthetic action spectrum for *A. nidulans* published by Kok and Hoch (10) which shows a major red drop. Biggins (1) was able to obtain both photosystems I and II activity in lysozyme treated cells of *Phormidium luridum*. We have been able to demonstrate only photosystem I activity in lysozyme treated cells of *Anacystis nidulans*. This may be explained in part by the use of a different blue-green alga in our experiments.

Action spectra for photosystems I and II in *Porphyridium cruentum* are shown in Fig. 2. Photosystem I peaks in the region of maximum chlorophyll a absorption with small peaks at 630 nm and in the region of phycocerythrin absorption (560 nm). Photosystem II action spectrum follows the absorption spectrum of phycocerythrin with a slight rise in the region of chlorophyll absorption. Our data agree with the electron transport scheme (13) for *P. cruentum* described by Nishimura. This scheme indicates that phycocerythrin is the main pigment involved in photosystem II with a minor role for chlorophyll a, while the latter is the major pigment for photosystem I.

The action spectra for photosystems I and II of formaldehyde fixed *Chlorella pyrenoidosa* as compared with its absorption spectrum are shown in Fig. 3. It can be seen that the action spectra generally follow the absorption spectrum of the alga except that photosystem II is more active in the region of chlorophyll b absorption (650 nm) than photosystem I. The action spectra generally agree with those of Joliot et al. (7) and Myers and French (12). However, our measurements on dye reduction by fixed cells are generally less reproducible than
polarographic measurements on unfixed material.

These data support the validity of formaldehyde fixed cells as models for studying photosynthetic electron transport. The ease of preparing fixed cells provides a useful method for studying photosystems I and II in algae.

**Literature Cited**


