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Role of Cytochrome and Pyridine Nucleotide in Algal Photosynthesis

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All known important biological oxidation-reduction catalysts, such as cytochromes, flavins, and pyridine nucleotides, show appreciable changes in the near-ultraviolet or visible region of the absorption spectrum when they undergo oxidation or reduction. If any of these catalysts take part in photosynthesis, one would expect their oxidation-reduction state to change upon illumination.

To study the changes in absorption spectrum that may occur in photosynthesizing cells, a sensitive absorption spectrophotometer was developed with which, for the first time, fast, reversible changes in absorption spectrum were observed in purple bacteria (*1–3*) and in *Chlorella* (*4*). In general the changes upon illumination and upon darkening happened within a few seconds. In purple bacteria the difference spectrum in the visible region, obtained by subtracting the absorption spectrum in the light from that in the dark, was similar to, but not identical with, the spectrum obtained by subtracting the absorption spectrum of oxidized from that of reduced cytochrome *c* (*3*).

These experiments showed that a cytochrome pigment was quickly oxidized in the light and reduced in the dark. In *Chlorella* a peak of the difference spectrum was found at 420 mμ (*4*). In analogy with our findings with purple bacteria, this peak was tentatively attributed to the oxidation in light of cytochrome *f*, a cytochrome pigment discovered by Hill and Scarisbrick (*5*), and only found in leaves and algae. However, there was also a much higher peak at 520 mμ and a smaller one at 480 mμ. Since neither of these peaks could be attributed to a cytochrome, the interpretation of the effect as a whole was uncertain. Lundegårdh (*6*), by using a modification of a flow method used by us before (*2*), measured a narrow region of the difference spectrum of *Chlorella* from 550 to 570 mμ and found, at 555 mμ, a small dip in the difference spectrum, which too, could be attributed to cytochrome *f* (and so interpreted by him).

The experiments described in this article (*7*) were performed with an apparatus similar in principle to but having a greater sensitivity and range than the original one (*1*).

Clear-cut evidence for the oxidation of a cytochrome in algae was obtained in experiments on the red alga *Porphyridium cruentum*. The difference spectrum is shown in Fig. 1. The spectrum shows a maximum at 555 mμ which occurs also in the difference spectrum of cytochrome *f*, shown in the same figure, and maximums in the blue resembling those of cytochrome *f*. This shows that in *Porphyridium* cytochrome *f*, or a cytochrome with similar absorption spectrum, becomes oxidized in the light and reduced in the dark. The spectrum of *Porphyridium* was measured after the cells had been in a closed vessel in the dark for about half a day, a treatment that was found to increase the change in absorption upon illumination without profoundly changing the shape of the spectrum in the visible region.

In contradistinction to the spectrum of *Chlorella*, the difference spectrum of *Porphyridium* did not show pronounced peaks at 480 and 520 mμ. The difference spectrum of *Chlorella* showed a peak at 420 and a small dip at 555 mμ, both of which were very probably caused by the same cytochrome pigment as the peaks at 420 and 555 mμ in *Porphyridium*. The magnitude and the time course of the absorption changes in the maximums at 420 and 520 mμ were influenced differently by changing the medium, indicating that these maximums resulted from two different substances.

The oxidation of a cytochrome in light and its reduction in dark in photosynthesizing species of widely different groups suggest an important role for this pigment in photosynthesis. In respiration, the reactions leading to the oxidation of DPNH (reduced diphosphopyridine nucleotide) are mediated by cytochrome *c*. In these reactions, ATP (adenosine triphosphate) is probably generated (*9*), and the function of the cytochrome in photosynthesis may well be that of an intermediate in processes leading to the formation of ATP, which presumably is needed to assist in the carbon dioxide reduction (*10*). Arnon *et al.* (*11*) showed that ATP can be generated by illuminated chloroplasts from added ADP (adenosine diphosphate) and inorganic phosphate.

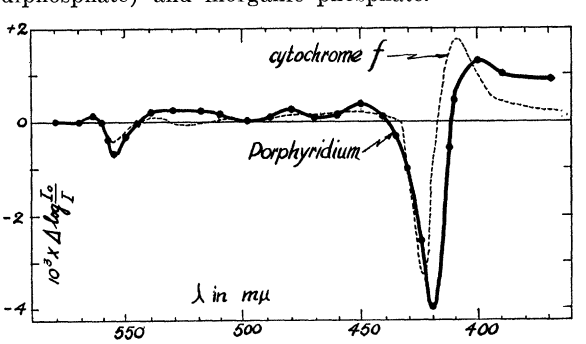

Fig. 1. Difference spectrum of *Porphyridium*, absorption spectrum in light minus that in darkness; and of cytochrome *f*, oxidized minus reduced. The latter spectrum was obtained by subtracting the spectra of reduced and oxidized cytochrome *f* measured by Davenport and Hill (*8*). The optical density of the *Porphyridium* suspension corrected for scattering was 0.46 at 680 mμ.

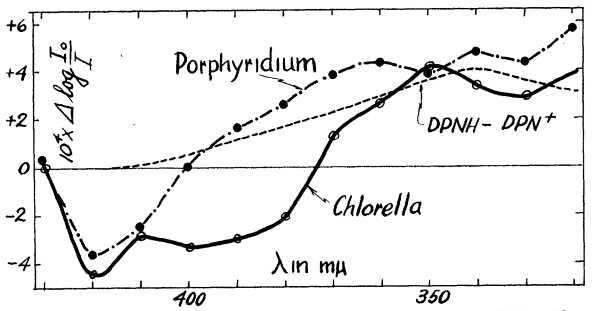

Fig. 2. Difference spectra of *Porphyridium* and *Chlorella*, light minus darkness, and difference spectrum of DPN. The optical density of the *Porphyridium* suspension corrected for scattering was 0.47 at 630 mμ, that of *Chlorella* 0.60 at 680 mμ.

The difference spectrum of *Chlorella* as well as that of *Porphyridium*, were measured for suspensions freshly prepared from growing cultures. Both spectra show an increase in absorption around 350 mμ (Fig. 2). The absorption of pyridine nucleotide increases upon reduction in the same region. Unfortunately the difference spectrum does not have several sharp peaks like the difference spectrum of the cytochrome pigments. Thus we can say only that the difference spectrums of the algae in the ultraviolet support the working hypothesis that upon illumination of photosynthesizing cells a pyridine nucleotide becomes reduced to be reoxidized within a few seconds in the dark.

The broadening of the peak at 420 mμ in the *Porphyridium* spectrum of Fig. 2, as compared with the corresponding peak in the spectrum of Fig. 1, may be caused by the different growth conditions or subsequent treatment of the culture. The decrease in absorption in the *Chlorella* spectrum around 390 mμ may be the result of the activity of the pigment or pigments that are responsible for the maximums at 480 and 520 mμ.

The optical density of the *Chlorella* suspension at 680 mμ corrected for scattering was 0.60. The measured optical density of chlorophyll and the change in optical density of pyridine nucleotide indicate that one molecule of nucleotide is reduced in light per about 100 chlorophyll molecules. When DPN^+ (diphosphopyridine nucleotide) was added to a final concentration of $10^{-4}M$ to a chloroplast suspension, in our apparatus a partial reduction of added DPN^+ could be observed. The ratio of reduced to oxidized form was about 1/2000. This explains why the reduction of DPN^+ by illuminated chloroplasts could not be detected before by conventional spectroscopy. This experiment shows, in a direct way, that DPN^+ can be reduced by illuminated chloroplasts, and confirms a conclusion previously reached in an indirect way (*12*).

The results of these experiments, combined with those of others, make plausible the following hypothesis, which can be tested by further experiments. Chlorophyll *a*, directly or indirectly excited by light, reacts with an oxidized pyridine nucleotide, PN^+, and an unknown oxidant, ZH, to give reduced pyridine nucleotide, PNH, and Z a strongly oxidizing compound. This reaction is followed by various dark reactions. Z is used for the greater part to oxidize water. Part of the Z oxidizes a cytochrome, presumably cytochrome *f*. The PNH also participates in two reactions. The main part is used for reduction of CO_2; a smaller part of the PNH is used for the reduction of the cytochrome (via intermediate enzymes). The oxidation of PNH by Z, mediated by the cytochrome, generates ATP to assist in the reduction of CO_2.

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Carbon Dioxide Fixation by Roots

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In the course of recent work we have had occasion to analyze the pattern of carbon dioxide fixation by roots, primarily the onion and soybean (*1*). Although this subject has been investigated (*2*) with barley roots, some differences in the distribution of radioactivity make it appear worth while to report our results.

Six to eight excised roots approximately 1 cm long were sealed in a vial containing 1 ml of distilled water and $NaHC^{14}O_3$ (0.125*M*). In a control experiment to test the possible effects of excision, onion roots about 10 cm long were passed through a glass tube bent at a 90° angle. The bend was sealed with mercury, permitting exposure of the roots alone to radiocarbon dioxide without removal of the roots from the bulb. The pattern of fixation of excised and attached roots proved to be identical. After 1 hr of exposure to the radioactive solution, the roots were dropped into boil-