the spectra of both PS 1 and PS 2 particles is more difficult to assign. In the PS 1 fraction derivative band f can be ascribed to chlorophyll *a* 680 which has an absorption maximum at about 678 nm at —196°. In the PS 2 fraction, band f is due to a pigment which appears as a small shoulder in the absorption spectrum between 680 and 685 nm. Derivative band f in the PS 2 particles thus appears to be due to small amounts of a PS 2-associated pigment with an absorption maximum near 684 nm, rather than to a contamination by PS 1. Derivative bands g and h are readily assigned to PS 1. In addition, there is a broad absorption band between 700 and 720 nm which is associated with the PS 1 particles.

Not all of the absorption bands found in spinach are ubiquitous to green photosynthetic systems. Low-temperature derivative spectra of Scenedesmus and Chlorella do not show bands a, c, e (data not shown).

We would like to thank Dr. T. YAMASHITA for help in the preparation of purified plastocyanin. This research was supported by National Institutes of Health Grant (GM 15048) to W.L.B. and a National Science Foundation postdoctoral fellowship to W.A.C.

Department of Biology, W. A. CRAMER
Revelle College University of California at W. L. BUTLER
San Diego, La Jolla, Calif. (U.S.A.)

1 J. M. ANDERSON AND N. K. BOARDMAN, *Biochim. Biophys. Acta*, 112 (1966) 403.
2 N. K. BOARDMAN, S. W. THORNE AND J. M. ANDERSON, *Proc. Natl. Acad. Sci. U.S.*, 56 (1966) 586.
3 B. KOK AND H. J. RURAINSKI, *Biochim. Biophys. Acta*, 126 (1966) 584.
4 J. M. ANDERSON, D. C. FORK AND J. AMESZ, *Biochem. Biophys. Res. Commun.*, 23 (1966) 874.
5 Y. F. FREI, *Biochim. Biophys. Acta*, 57 (1962) 82.
6 L. P. VERNON, *Anal. Chem.*, 32 (1960) 1144.
7 W. L. BUTLER AND N. I. BISHOP, *Photosynthetic Mechanisms in Green Plants, Natl. Acad. Sci.–Natl. Res. Council Publ.*, 1145 (1963) 91.
8 W. L. BUTLER, *Biochim. Biophys. Acta*, 102 (1965) 1.
9 C. S. FRENCH, *Brookhaven Symp. Biol.*, 11 (1958) 65.
10 C. S. FRENCH, *Biochemistry of Chloroplasts*, Academic Press, New York, 1966, p. 377.
11 J. B. THOMAS, *Biochim. Biophys. Acta*, 59 (1962) 202.
12 H. METZNER, *Microalgae and Photosynthetic Bacteria*, University of Tokyo Press, Tokyo, 1963, p. 227.

Received January 22nd, 1968

BBA 43195

Light-induced spectroscopic changes in the 600-mμ region in leaves of a higher plant and in Chlorella

We have previously observed[1] light-induced absorbance changes in the 600-mμ region in green plants and algae which were attributed to variations in the redox state of the copper–protein plastocyanin discovered by KATOH *et al.*[2]. In these experiments we have made measurements *in vivo* on leaves of a higher plant and on

Chlorella. In spite of the fact that the changes around 600 mμ are often small, rather slow, and complicated by other changes, the difference spectra in this region suggest that there is a contribution of a specific substance, which may be plastocyanin. In addition, measurements of absorbance changes with the sample within an integrating sphere showed that the possible contribution of light-induced scattering changes to our measurements in the 600-mμ region was negligible.

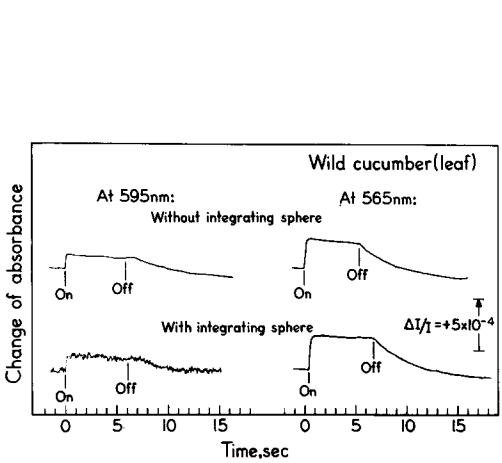
Light-induced changes of absorbance were measured on *Chlorella pyrenoidosa* in an open cuvette or by placing leaves of wild cucumber, *Marah* (*Echinocystis*) *fabaceus*, on a lucite light pipe above the photomultiplier (EMI 9558B) of the apparatus described previously[1]. Since the samples were placed directly on top of the light pipe, a large angle of scattered light was reflected into the photomultiplier and the apparatus was thus relatively insensitive to light-induced changes of scattered light which would appear as light-induced absorbance changes. In order to differentiate more carefully between light-induced scattering changes and true absorbance changes, the measurements were repeated but with the same sample held inside an integrating sphere having a diameter of 25 cm. With this arrangement, scattered light eventually reaches the photomultiplier by multiple reflections within the sphere. Thus a scattering change will disappear or be greatly diminished when the sample is inside the sphere but a light-induced absorbance change will be unaffected (except for increased "noise" caused by a higher voltage applied to the photomultiplier).

Fig. 1 shows examples of light-induced absorbance changes at 565 and 595 mμ made with leaves of wild cucumber inside and outside the integrating sphere. Since the sphere had no effect on these changes, we concluded that the light-induced increases of absorbance at these two wavelengths represent true absorbance changes. The intensities, wavelengths and band widths of the measuring beam and the actinic beams were kept unchanged for the two sets of experiments (*i.e.* inside and outside the sphere). The possible contribution of chlorophyll fluorescence or of fluorescence of the filters placed over the photomultiplier was checked and found to be undetectable. (Fluorescence was determined with the measuring beam turned off.)

Fig. 1 shows that the steady-state absorbance changes are larger at 565 mμ than at 595 mμ and that the immediate dark "decay" of the absorbance change following far-red illumination is quite different depending on wavelength. Actually, an immediate decay is seen at 565 mμ but at 595 mμ there is first a slow increase of absorbance upon turning off the light and only afterwards is a decay seen.

We have determined the slowly "decaying" component by plotting $V_1/\Delta S$ as a function of wavelength (where V_1 is the initial off-rate and ΔS the steady-state deflection). The resulting spectrum (Fig. 2), measured in the sphere, was compared with the oxidized-*minus*-reduced spectrum of purified spinach plastocyanin[3]. In order to make this comparison possible, KATOH's spectrum was replotted by measuring the relative slopes of the absorption band at 5-mμ intervals, multiplying by an appropriate constant factor and replotting the absorption curve with these corrected values. This allows one to trace the spectrum starting from the maximum without needing to know the actual peak height in actual absorbance units. The *in vivo* $V_1/\Delta S$ spectrum, if due to a unique substance, even with several absorption bands, would give an horizontal line. If other substances with different kinetic characteristics are present, they will distort this line, giving more or less pronounced maxima or minima which would nearly correspond, as a first approximation, to their absorption bands.

Of course comparisons of $V_1/\Delta S$ spectra with true absorption spectra must be interpreted with care: a correspondence between the two spectra allows a tentative attribution of the $V_1/\Delta S$ band to a particular substance, but the absence of a correspondence does not rule out a correlation because there may be interference by other substances having much different kinetics or large overlapping of their absorption bands. The $V_1/\Delta S$ spectrum shows some correspondence to KATOH's plastocyanin curve plotted as described above. However, the spectrum of the cucumber leaf is broader than that of plastocyanin, especially on the short-wavelength side and is shifted about 5 mμ to shorter wavelengths. It is clear that there is interference with neighboring absorbance changes such as the one near 565 mμ which occurs in leaves.



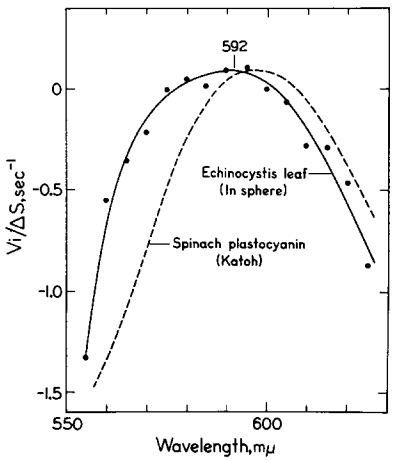


Fig. 1. Kinetics of light-induced absorbance changes at 595 and 565 mμ measured with a leaf of wild cucumber (*M. fabaceus*) within and without an integrating sphere. Half-band width of the measuring wavelength was 5 mμ. An upward deflection corresponds to an increase of absorbance. Incident actinic light (on and off arrows) was 709 mμ, 10200 erg (= 6 nanoEinstein)$\cdot$cm$^{-2}\cdot$sec^{-1}. Room temperature (22°), air. Notice the difference in the off-kinetics at 565 and 595 mμ.

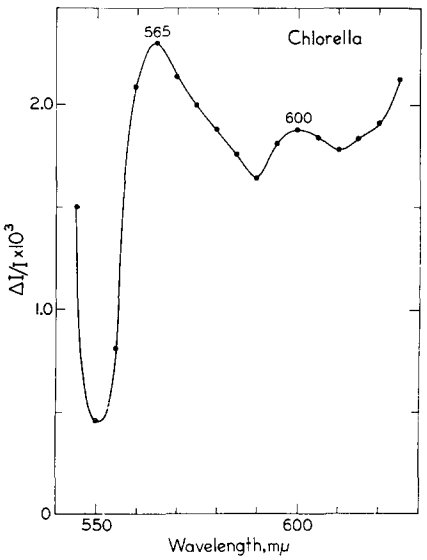
Fig. 2. Spectrum (mean values of 4–6 consecutive measurements at each wavelength) for the slowly "decaying" absorbance change following 709-mμ actinic light (same intensity and conditions as for Fig. 1) in a leaf of wild cucumber. The measurements were made with the sample in an integrating sphere. The ordinate is the relative "rate constant" ($V_1/\Delta S$, see text) of the change when actinic light is turned off. A computed oxidized-*minus*-reduced spectrum of spinach plastocyanin is plotted for comparison (see text).

This change is probably produced in part by a *b*-type cytochrome but another substance appears to contribute to this change.

The difference spectrum in Chlorella for the steady-state absorbance change (ΔS) is given in Fig. 3. In Chlorella the changes near 565 mμ are smaller in relation to those near 600 mμ than in leaves. The spectrum of the slowly "decaying" component of Chlorella was determined as described for leaves and is given in Fig. 4.

As pointed out above, light-induced absorbance changes caused by plastocyanin are often difficult to detect for several reasons. The extinction coefficient of plastocyanin is low, only about half of that of the α-band of cytochrome *f*. Moreover, the absorbance band of the oxidized-*minus*-reduced spectrum of plastocyanin is very broad with a half-band of about 100 mμ. Also, in chloroplast preparations, part of

the plastocyanin may be washed out during extraction. Finally, depending on the organism, the absorption changes caused by plastocyanin can be masked by other large changes in nearby spectral regions as was shown here for Marah and Chlorella.



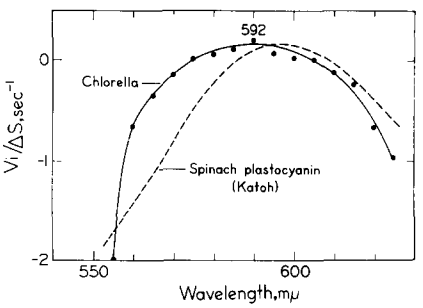


Fig. 3. Light-*minus*-dark difference spectrum for the steady-state absorbance change produced in *C. pyrenoidosa* by 709-mμ actinic light (same intensity and conditions as for Fig. 1). The half-band width of the measuring wavelength was 4 mμ.

Fig. 4. Spectrum for the slowly "decaying" absorbance change following 709-mμ actinic light with Chlorella (same intensity and conditions as for Fig. 1). The ordinate is the relative "rate constant" as in Fig. 2. The oxidized-*minus*-reduced spectrum of spinach plastocyanin is plotted for comparison.

Although it is difficult to prove with certainty that the light-induced absorbance changes in the 600-mμ region are really due only to plastocyanin, there are enough suggestions that this copper protein may contribute to the observed spectroscopic phenomena.

Department of Plant Biology, DAVID C. FORK
Carnegie Institution of Washington,
Stanford, Calif. 94305 (U.S.A.)

Laboratoire de Photosynthèse, C.N.R.S., YAROSLAV DE KOUCHKOVSKY
91. Gif-sur-Yvette (France)

1 Y. DE KOUCHKOVSKY AND D. C. FORK, *Proc. Natl. Acad. Sci. U.S.*, 52 (1964) 232.
2 S. KATOH, I. SUGA, I. SHIRATORI AND A. TAKAMIYA, *Arch. Biochem. Biophys.*, 94 (1961) 136.
3 S. KATOH, I. SHIRATORI AND A. TAKAMIYA, *J. Biochem.*, 51 (1962) 32.

Received January 8th, 1968