A FLAVIN-CHLOROPHYLL a COMPLEX RESEMBLING THE P700 OF PHOTOSYNTHESIS

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SUMMARY

A model molecular complex of chlorophyll a and FMN is found to have properties resembling the P700 of photosynthesis. The model complex shows an absorption maximum near 700 nm which can be bleached reversibly by light. ESR studies show that the photobleaching is accompanied by light-driven electron transfer from the chlorophyll a to the flavin. These observations are consistent with the previous suggestion that P700 may be a molecular complex of chlorophyll a and the prosthetic group (FAD) of chloroplast $NADP^+$-reductase.

The discovery of the reversible photobleaching of purple bacteria by Duysens (1952) stimulated the growth of exciting research on the light-sensitive reaction centers in both bacterial chromatophores (Duysens, 1953, 1958; Thomas and Goedheer, 1953; Goedheer, 1958; Clayton, 1962; Nicolson and Clayton, 1969; Ke, 1969) and the chloroplasts of green plants (Kok, 1961; Witt et al., 1961; Kok and Hoch, 1961; Müller et al., 1963). Although the observed bleaching has generally been attributed to the reversible oxidation of a particular bacteriochlorophyll (P890 or P870) or chlorophyll a (P700) molecule in a special environment, the molecular composition of the special environment remains unspecified.

In view of the conclusion that the primary function of P700 is to utilize light energy to drive electrons from a donor to $NADP^+$ and that previous experiments in model systems (Vernon et al., 1965; Tu and Wang, 1969) suggest the sequence of this electron transfer to be donor → chlorophyll a → $NADP^+$-reductase → $NADP^+$, a working hypothesis has recently been proposed by assuming P700 to be a particular chlorophyll a molecule

which is complexed with the flavin group of $NADP^+$-reductase (Wang, 1969). The present work demonstrates that chlorophyll a and flavin mononucleotide (FMN) indeed form a molecular complex with properties resembling those of P700.

MATERIALS AND METHODS

Chlorophyll a and methyl chlorophyllide a were prepared from fresh spinach and purified by chromatography on a sucrose column by the method of Holt and Jacobs (1954). FAD, FMN and NADH were obtained from Sigma Chemical Co.

Bleaching experiments were monitored with a Warner and Swasey Model 501 Rapid Scanning Spectrometer, with scanning time set at 12.5 msec for the spectral range 555 to 795 nm. A Sylvania Sun Gun shielded with a 2-inch thick cold water filter was used as the actinic light source. The EPR data were obtained by means of a Varian E-3 EPR Spectrometer. The reaction mixtures were sealed under high vacuum in quartz cells fused onto Pyrex glass manifolds.

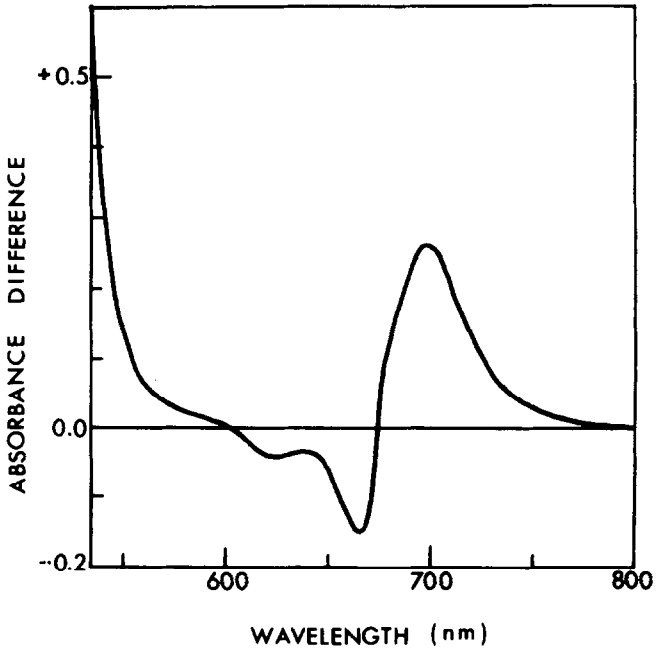


Fig. 1. Difference spectrum of FMN-chlorophyll a complex against chlorophyll a . Solution in sample cell: [total FMN] = 2.46 X 10^{-2} M, [total chlorophyll a] = 9.2 X 10^{-5} M, in acetone-water (15:85) mixture containing 0.01 phosphate buffer at pH 7.0 and 23°C. Solution in reference cell: Same as the solution in the sample cell but without FMN. (The reference solution was used within a few minutes after it was prepared.)

RESULTS

The difference absorption spectrum of chlorophyll a in the presence of an excess of FMN against chlorophyll a alone in acetone-water solution (15% acetone, v/v) at pH 7 is shown in Fig. 1. Since FMN does not absorb appreciably above 600 nm, this spectrum must be due to the difference in absorbances of the FMN-chlorophyll a complex and chlorophyll a with their absorption maxima at ~700 nm and 670 nm respectively.

The results of a reversible photobleaching experiment are shown in Fig. 2. A tungsten lamp with blue filter was used as the scanning light.

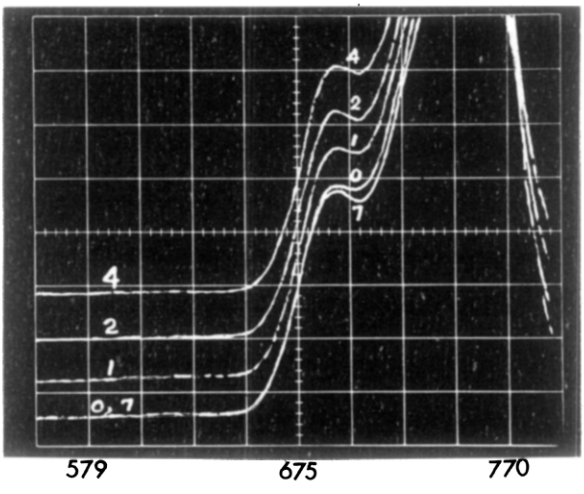


Fig. 2. Intensity versus wavelength profile of a FMN + chlorophyll mixture at different stages of the photobleaching experiment. The number on each curve denotes the time in min. after the actinic light was turned off when the 12.5-msec scan was made. The ordinate represents light intensity (see text) and the abscissa represents wavelength in nm. Composition of solution: [FMN] = 2.7×10^{-2} M, [chlorophyll a] = 6.3×10^{-5} M in acetone-water (15:85) solution containing 0.01 M phosphate buffer at pH 7.0 and 23°C.

For the convenience of visual examination, the intensity versus wavelength profiles of the transmitted scanning light at 0, 1, 2, 4 min. respectively after the steady actinic light was turned off were displayed at equal vertical separations on the oscilloscope screen. The profile at 7 min. after the actinic light was turned off was found indistinguishable from the profile before bleaching by the actinic

light, and was adjusted for easy comparison to have its base line coinciding with that at 0 min. When these data were replotted on the absorbance scale, it was found that maximum bleaching occurred near 705 nm. For the photobleaching experiments where chlorophyll a was replaced by methyl chlorophyllide a the results were qualitatively similar, but the observed recovery time was shorter by a factor of 10^3.

Although the above FMN + chlorophyll a mixtures showed no detectable EPR signal in the dark, under steady actinic light the mixture exhibited at 23° and -150°C respectively the EPR spectra shown in C and D of Fig. 3. A com-

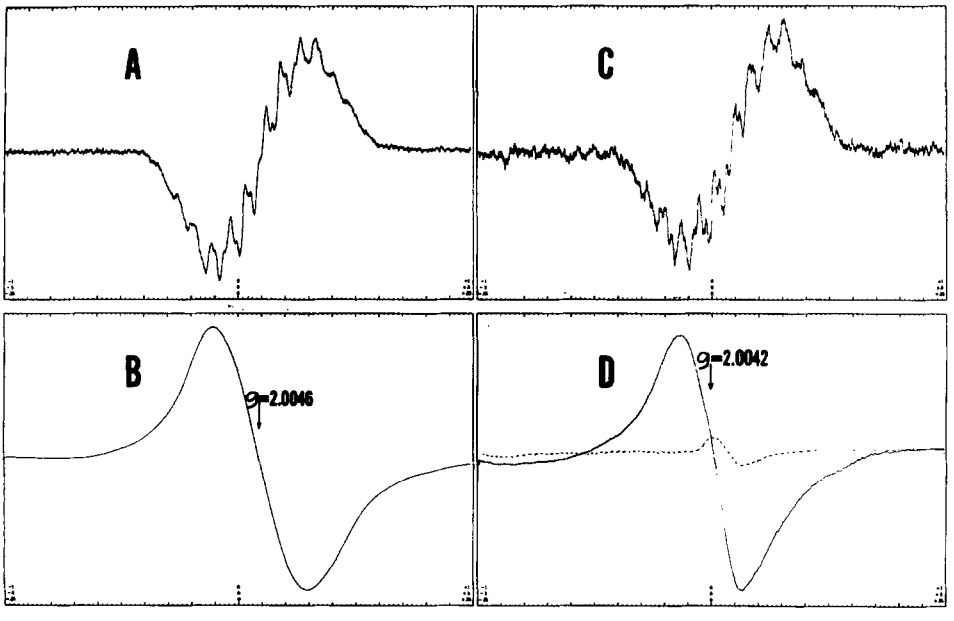


Fig. 3. EPR spectra of model systems.

(A) FMN + NADPH (1:4) in aqueous 0.01 M phosphate buffer at pH 7.0 and 23°C, in the dark. Magnetic field set at 3370 G; scan range, 100 G; microwave frequency 9.533 GHz.

(B) FMN + NADPH (1:10) in aqueous 0.01 M phosphate buffer at pH 7.0 and -150°C, in the dark. Magnetic field set at 3230 G; scan range, 100 G; microwave frequency 9.145 GHz. Observed half-width of EPR signal = 20 G.

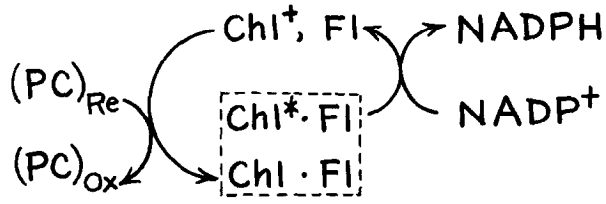
(C) FMN (2.5×10^{-2} M) + chlorophyll a (1.9×10^{-4} M) in acetone-water solution containing 0.01 M phosphate buffer at pH 7.0 and 23°C, under steady actinic light. Magnetic field set at 3370 G; scan range 100 G; microwave frequency, 9.531 GHz.

(D) Same composition as in (C) but at -150°C, under steady actinic light. Magnetic field set at 3235 G; scan range 100 G; microwave frequency, 9.146 GHz. Observed half-width of EPR signal = 15 G. The broken curve represents the EPR signal of the empty quartz sample cell at this particular instrument setting.

parison of C and D with the EPR spectra of the flavin radical in FMN + NADPH mixtures in the dark at the same temperatures (A and B of Fig. 3) indicates that light-driven electron transfer from chlorophyll a to FMN has occurred in C and D.

DISCUSSION

The above experimental results suggest that P700 may be a molecular complex (Chl·Fl) of a particular chlorophyll a molecule (Chl) and the flavin group (Fl) of chloroplast $NADP^+$-reductase. In such a hypothetical model, this complex could utilize light energy to drive electrons from a donor such as plastocyanine (PC) to $NADP^+$ by the following mechanism:



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