P 700 AND CYTOCHROME F IN PARTICLES OBTAINED BY DIGITONIN FRAGMENTATION OF SPINACH CHLOROPLASTS

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Boardman and Anderson (1964) reported that incubation of spinach chloroplasts with digitonin caused a fragmentation into particles of different sizes, which could be separated by centrifugation. In view of the pigment composition, the photochemical activities and the manganese content of these particles (Boardman and Anderson, 1964; Anderson et al., 1964), they postulated that digitonin resulted in a physical separation of the two pigment systems of photosynthesis (systems 1 and 2 cf. review by Vernon and Avron, 1965). "Large particles" sedimentable at 10,000 x g were enriched in system 2, and the "small particles" sedimentable at 144,000 x g were representative of system 1. Experiments on light-induced changes in optical density in the region around 700 mμ reported in this paper, indicate that small particles contain approximately twice as much P 700 as untreated chloroplasts and three to four times more than the large particles. As there is evidence that P 700 acts as the reaction center of system 1 (Kok and Hoch, 1961), this indicates that a partial separation of the two photochemical systems of photosynthesis has been achieved by digitonin fragmentation.

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Measurements of absorption changes in the blue region of the spectrum were in agreement with this conclusion.

After incubation of spinach chloroplasts for 30 min at 0° with 0.5% digitonin (Boardman and Anderson, 1964), the chlorophyll-containing particles were separated by differential centrifugation at the following speeds; 1000 x g for 10 min, 10, 000 x g for 30 min, 50, 000 x g for 30 min and 144, 000 x g for 60 min. The sediments were resuspended in a phosphate -KCl buffer. The light-induced changes of optical density were recorded by means of an apparatus previously described (de Kouchkovsky and Fork, 1964).

In all fractions changes of optical density around 700 mμ were observed upon illumination. In order to measure the maximum amount of P 700 bleached upon illumination, it was necessary to apply a high intensity of actinic light and to keep the intensity of the measuring beam low. Since the high actinic intensity caused strong chlorophyll fluorescence, the photomultiplier was placed 30 cm from the cuvette in order to minimize selectively the fluorescence signal. Changes of optical density were corrected for fluorescence by subtraction of the signal obtained when the measuring beam was shut off. Since the intensity of the measuring beam was at least 10^4 times lower than that of the actinic light, errors caused a change of yield of fluorescence excited by the measuring beam were assumed to be neglible. Fig. 1 shows difference spectra of the 144, 000 x g and 10, 000 x g fractions obtained in the presence of reduced DAD (2, 3, 5, 6-tetramethyl-p-phenylenediamine), which acts as an effective electron donor for system 1 (Trebst and Pistorius, 1965). The spectra showed maxima at 702 and 683 mμ indicating the oxidation of P 700 and possibly the bleaching of a pigment absorbing at 683 mμ. Since a realtively large fluorescence signal was observed at 683 mμ especially with the 10, 000 x g fraction, the measurements in

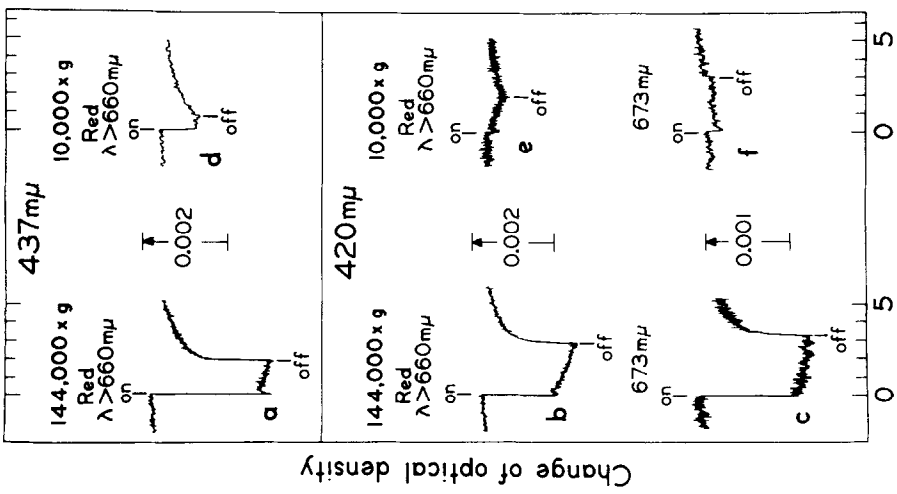

Fig. 2. Light-induced changes of optical density at 437 and 420 mμ for the 144,000 x g fraction (140 μg chlorophyll/ml) and the 10,000 x g fraction (184 μg chlorophy/ml) with 8.3×10^{-5} M DAD and 1.2×10^{-3} M sodium ascorbate. The actinic light used for upper traces (a), (b), (d) and (e) had an intensity of 10^5 ergs cm^{-2} sec^{-1} between 650 and 700 mμ, while for traces (c) and (f) the monochromatic light at 673 mμ had an intensity of 2.8×10^4 ergs cm^{-2} sec^{-1}.

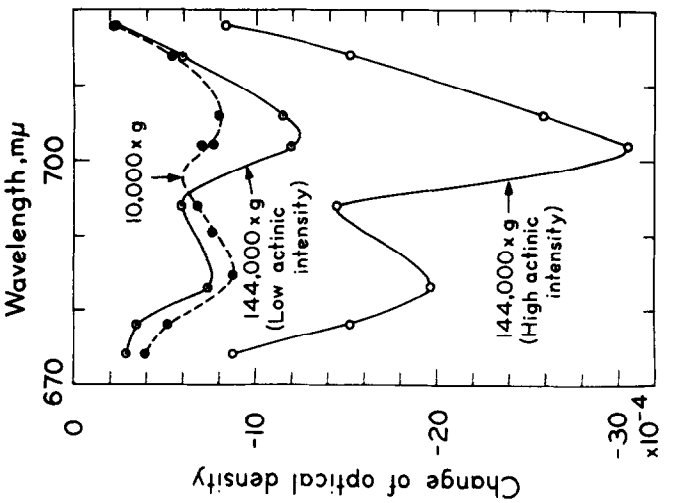

Fig. 1. Difference spectra for the 144,000 x g fraction (134 μg chlorophyll/ml) activated by blue light of high (6×10^4 ergs cm^{-2} sec^{-1}) and low (4×10^3 ergs cm^{-2} sec^{-1}) intensity respectively, and for the 10,000 x g fraction (144 μg chlorophyll/ml) activated by a light intensity of 3×10^4 ergs cm^{-2} sec^{-1}. The measurements were made in the presence of 8.3×10^{-5} M DAD and 1.2×10^{-3} M sodium ascorbate.

this area may be less reliable.

The amount of P 700 in the various fractions and in chloroplasts fragmented by means of a French press was calculated from the decrease of optical density at 702 mμ upon illumination with blue light of saturating intensity. A specific extinction coefficient of 82 l g^{-1} cm^{-1} for P 700 at 702 mμ was applied, the same as that of chlorophyll a in 80% acetone at 663 mμ (MacKinney, 1941). The chlorophyll content of the fractions was calculated after extraction with 80% acetone (Arnon, 1949).

Table 1 shows that the highest ratio of P 700 was found in the 144,000 x g fraction (one P 700 for about 200 chlorophyll molecules). In the 10,000 x g

Table 1. Comparison of the relative amounts of chlorophyll to P 700 for chloroplasts fragments and for the centrifugal fractions obtained by digitonin fragmentation, in the presence of 8×10^{-5} M DAD and 1.2×10^{-3} M sodium ascorbate.

Fraction	Chlorophyll a and b / P 700	Chl a / Chl b
Chloroplast fragments	460	2.82
	420	2.82
10,000 x g	650*	2.31
	730	2.40
50,000 x g	330	4.10
144,000 x g	235	5.42
	175	5.88
144,000 x g supernatant	980	3.86

* With 1.2×10^{-5} M N-methylphenazonium methosulphate instead of DAD

fraction there was only one P 700 for every 700 chlorophylls. The chlorophyll to P 700 ratio for untreated chloroplasts is in agreement with the value reported

by Kok (1961). The calculated chlorophyll to P 700 ratio obtained by summing individually the chlorophyll and P 700 contents of the fractions, was 420.

These results are in agreement with the hypothesis that digitonin fragmentation of chloroplasts brings about a partial separation of systems 1 and 2. This was also indicated by measurements of absorption changes in the blue region. The difference spectrum had a maximum at 425 mμ which indicated overlap of P 700 (430 mμ maximum: Kok, 1961; Witt et al., 1965) and cytochrome f oxidation (420 mμ maximum: Duysens, 1955); a positive change at 405 mμ confirmed that cytochrome f was being oxidized. Fig. 2, traces (a) and (d) show the kinetics of the absorbance change at 437 mμ, which indicate in agreement with the results at 702 mμ, that little P 700 is present in the 10,000 x g fraction. The lower traces (b), (c), (e) and (f) indicate that there is relatively very little oxidation of cytochrome f in the 10,000 x g fraction as compared to the 144,000 x g fraction, at both saturating and low light intensities. It should however, be noted that the change of optical density at 420 mμ in both fractions is lower than would be expected if P 700 and cytochrome f were present in equal amounts.

Our experiments are evidence for the actual separation of system 1 from system 2, rather than an inactivation of system 2 in the small particles. If it is assumed that P 700 is associated with system 1, and that the chlorophylls are approximately evenly divided between system 1 and 2, the ratio of chlorophyll to P 700 would be expected to be about 200 for system 1 particles. This value was actually found for the 144,000 x g fraction. The figure of one P 700 for about 700 chlorophyll molecules in the 10,000 x g fraction would indicate that these particles contain about 70% of system 2 and 30% of system 1, in good agreement with the estimation based on the photochemical activities (Anderson and Boardman, 1966).

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