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Determination of the concentration and the redox potential of chloroplast cytochrome 559

Incubation of isolated spinach chloroplasts for 10 min with buffered Triton X-100 (1.0 g Triton X-100 per 0.06 g chlorophyll) leads to solubilization of Photosystem I and cytochromes b_6 and f (ref. 1), which can be separated by centrifugation at 30000 × g. The residue, which is here denoted 30P10 fraction, contains cytochrome 559 but is usually devoid of cytochromes b_6 and f. Assay of the cytochromes is achieved by stepwise reduction from the fully ferricyanide-oxidized condition, with concomitant observation of the difference spectrum in the α-band region. Our technique, however, differs from that of other workers[1-4] in the use of crystalline hydroquinone as an intermediate reductant. Hydroquinone, with E_h = +230 mV at pH 7.0 (assuming a 1% quinone content), reduces cytochrome f (E_{m7} = +365 mV) but not cytochrome 559. Cytochrome 559 can then be estimated by addition of sodium ascorbate (E_h = 0 mV at pH 7.0 assuming a 1% dehydroascorbate content). Dithionite is added finally to reduce cytochrome b_6. Fig. 1 shows difference spectra obtained in this way.

The successive fractionation of Photosystem I and the cytochromes by differential centrifugation is presented in Table I, from which it is clear that the intermediate titration with hydroquinone is essential in the early stages of fractionation, as long as significant quantities of cytochrome 559 remain in association with the light particle. If the 220P8 fraction (sedimenting after 8 h at 220000 × g) is subjected to sucrose density gradient fractionation, an upper band containing cytochromes b_6 and f is obtained and, in agreement with data of VERNON *et al.*[1], this is found to be completely free of cytochrome 559.

In a short note discussing the E'_0 values of pea chloroplast cytochromes, BENDALL[5] states that the E'_0 of cytochrome 559 is +370 mV. A component with this characteristic potential should be reduced by hydroquinone and yet in our experience hydroquinone does not reduce this cytochrome in either the 30P10 fraction or in untreated chloroplasts. Accordingly, a determination of the cytochrome 559 midpoint potential was made using the 30P10 fraction, which offers advantages over

TABLE I

FRACTIONATION OF CHLOROPLASTS IN TRITON SOLUTIONS

30P10, 30P60 = pellets from centrifugation for 10 min and 60 min at 30000 × g; 220P8 = pellet from centrifugation for 8 h at 220000 × g; 220S8 = supernatant above 220P8.

Fraction	*Chlorophyll (mg)*	*Chlorophyll a / Chlorophyll b*	*Cytochrome f (nmoles)*	*Cytochrome 559 (nmoles)*	*Cytochrome b_6 (nmoles)*
30P10	25.3	1.87	0	96	0
30P60	24.3	1.90	11	112	9
220P8	37.4	4.95	119	36	190
220S8	27.4	1.58	8	44	31
Total	114.3	2.8 (Ratio)	138 1	288 2.1	230 1.7

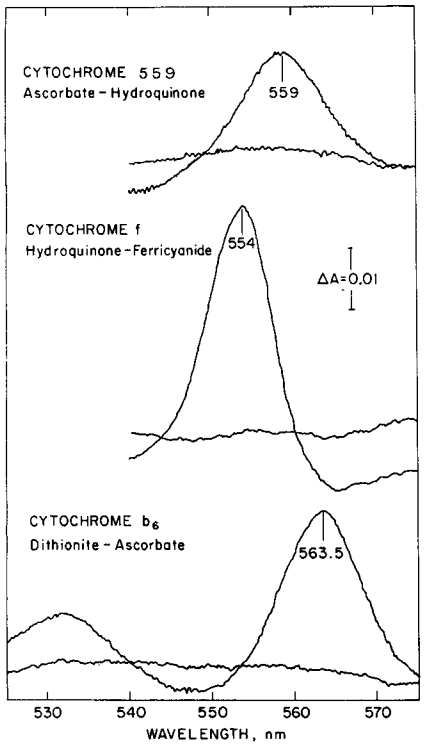

FIGURE 1

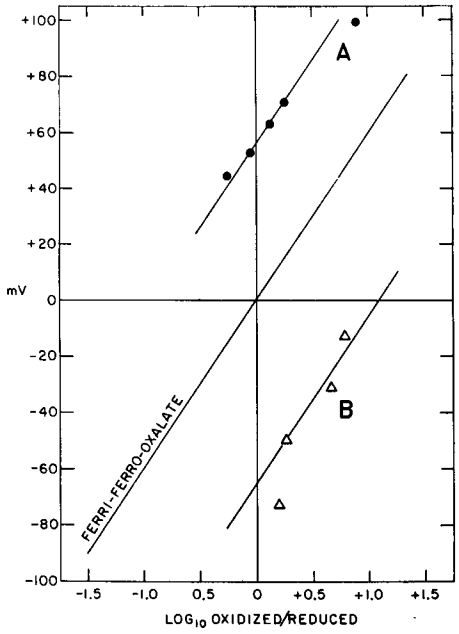

FIGURE 2

Fig. 1. Difference spectra (with corresponding baselines) of cytochromes in Triton-resolved chloroplast fractions. The fractions used were: 30P10 for cytochrome 559 and 220P8 for cytochromes b_6 and f (see Table I legend). Hydroquinone was purified by recrystallization from methanol. Samples were incubated for 10 min with reagents before recording spectra.

Fig. 2. Determination of the E_{m7} of cytochrome 559 by equilibration with ferri-/ferro-oxalate mixtures. A, untreated 30P10 fraction; B, acetone-extracted 30P10 fraction. The theoretical line for the potential of the ferri-/ferro-oxalate systems is also shown[12]. Titrations were performed as described by VELICK AND STRITTMATTER[13] except that the entire difference spectrum was recorded after each addition. Solid sodium dithionite and potassium ferricyanide were used to determine the maximum ΔA. Buffer: 0.1 M potassium oxalate, 0.01 M *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)[14], pH 7.0

untreated chloroplasts, namely: (a) freedom from other cytochromes, (b) increased cytochrome 559 to chlorophyll ratio, and (c) decreased light scattering. The results of an anaerobic titration with the ferri-/ferro-oxalate couple are shown in Fig. 2A, which gives $E_{m7} = +55$ mV (reported earlier[6]). If, however, the 30P10 fraction is freed from chlorophyll by extraction with 80% acetone at $-20°$, the E_{m7} drops to about -65 mV (Fig. 2B).

Studies were made on the effect of CO on the spectrum of reduced cytochrome 559 in the 30P10 fraction before and after acetone treatment. No significant weakening or shifting of the 559-nm band resulted from exposure to CO; although such changes could be readily observed following denaturation by strong alkali (pH 13). Apparently the effect of acetone extraction resembles the mild denaturation of cytochrome c, which leads to a negative shift in the E_{m7} but not to CO-binding ability[7].

The value of +55 mV reported above for the E_{m7} of cytochrome 559 is in reasonable agreement with that of +90 mV obtained by FAN AND CRAMER[8], using unfractionated chloroplasts, but not with the +370 mV reported by BENDALL[5]. In comparing the former two estimates it should be noted that resolution of chloroplasts with detergent may modify the environment of the cytochrome haem and hence the E_{m7}. On the other hand, equilibration of a pigment with known redox systems such as ferri-/ferro-oxalate avoids complications resulting from liquid junction potential errors possibly associated with direct electrometric titration.

The revised values for the midpoint potential of cytochrome 559 make conceivable the operation of this pigment as an electron transfer component between the two light reactions in photosynthesis, as has been suggested[9–11].

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