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Simultaneous Quantitative Comparison of the Optical Changes at 700 nm (P700) and Electron Spin Resonance Signals in System I of Green Plant Photosynthesis[1a,2]

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Abstract: The technique of simultaneous optical and electron spin resonance spectroscopy has been used to show that the ratio of light-induced spins in the esr signal I to bleached P700 species in spinach subchloroplast particles is 1.1 ± 0.2 and in whole chloroplasts is 1.2 ± 0.3. Thus within experimental error the concentration of $P700^+$ entities and the concentration of spins in the esr signal I are the same. Combined with other studies these results provide very strong evidence in favor of the hypothesis that the optical bleaching at ~700 nm and the light-induced esr signal I are physical manifestations of the same molecular component.

In 1956[3] Kok first observed, in algae and chloroplasts of green plants, a small reversible light-induced bleaching at ~700 nm (which he called P700). In 1962 Beinert, Kok, and Hoch[4] tentatively associated this bleaching with a light-induced esr signal (now called signal I). This esr signal had earlier been discovered by Commoner, Heise, and Townsend.[5] Although subsequent work[6] has given conflicting evidence as to whether or not these two physical attributes are manifestations of the same molecular species, the original hypothesis has not been disproven.[7]

Similar phenomena occur in bacterial photosynthesis and there appears to be no doubt that the light-induced esr signal and a photobleaching at ~870 nm are due to the primary photochemistry which consists of an electron transfer from a bacteriochlorophyll species (P870) to an acceptor which apparently contains Fe^{3+} within a single protein.[8-11] The situation in green plant or algal systems is not nearly so clear.

As discussed above, the behaviors of the optical species P700 and the esr signal I are similar. Although kinetic correspondence between P700 and signal I was claimed by Vernon, Ke, and Shaw,[12] the esr spectrometer time resolution was inadequate to justify a positive identification. Likewise, quantitative comparisons have given conflicting results. Beinert and Kok[13] were unable to demonstrate the numerical equivalence of spins and optical species in chloroplasts. Nevertheless, more recently Vernon, *et al.*,[14] and Weaver and Weaver[15] have both indicated that the spins/P700 ratio is near unity in subchloroplast preparations.

We have recently reported in this journal[16] that the kinetic responses of P700 and signal I are virtually identical in experiments conducted using simultaneous optical and electron spin resonance (SOESR) detection. We are now reporting on the fact that these two phenomena are quantitatively identical as well.

SOESR measurements were made on various preparations of subchloroplast particles and also on broken and intact chloroplasts. Photosystem I subchloroplast particles were prepared by detergent fractionation with Triton X-100[17] or digitonin.[18] Digitonin and Triton subchloroplast particles are designated D144 and TSF1, respectively. The preparation procedure for chloroplasts was similar to that reported by Yamashita and Butler.[19] The monitoring wavelength was generally 703 nm for P700 analysis with a passband of 3 nm. Monitoring light intensities were usually on the order of 100 erg cm^{-2} sec^{-1}. Sample concentrations were adjusted to provide an absorbance of 0.3–0.5 at 700 nm for an optical pathlength of 0.35 mm.

The output of the SOESR apparatus[20] was coupled to two channels of a Fabritek 1072 computer of average transients (manufactured by Nicolett Corp.). The digital output of the Fabritek 1072 was calibrated in

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(2) The work described in this paper represents part of the Ph.D. Thesis of Dr. J. T. Warden, Jr., University of Minnesota, 1972.

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(20) A complete description of the apparatus will be published later: J. T. Warden, Jr., and J. R. Bolton, manuscript in preparation; J. T. Warden, Jr., Ph.D. Thesis, University of Minnesota, 1972.

absorbance units by comparison with sample absorbances measured by a Cary 14 spectrophotometer.

Quantitation of the transient esr signals was accomplished by comparison with a standard $CuSO_4$ aqueous solution (0.9833×10^{-2} M) and 4-(*N*-maleimido)-2,2,6,6-tetramethylpiperidine nitroxide solutions. Spectra of the concentration standards were recorded by the CAT under experimental conditions which were identical with the unknown as to microwave power, modulation amplitude, temperature, solvent and sample geometry, and container. Only the scan width and amplifier gains were different for standard and unknown. Area determinations for the standards were effected by numerical double integration, either manually or by computer. A reproducibility of 5% in concentration determinations could usually be achieved, even under unfavorable experimental conditions (*e.g.*, darkness and the modified optical transmission cavity).

Area calculations for the unknown were obtained by two procedures. The first method assumed a gaussian line shape for signal I and allowed calculation of the area from the amplitude of the transient response monitored at either derivative maximum. Under these conditions, the area of the unknown esr signal is given by[21]

$$A = (2\pi e)^{1/2}(\tfrac{1}{2}\Delta H_{pp}^{2})y_m'$$

ΔH_{pp} represents the peak-to-peak line width of the first derivative curve and y_m' is the amplitude of the signal at a derivative extremum. The second method utilized a rapid field-scan unit to generate the spectrum of the decaying transient. The area was then determined by either manual or computer integration. Appropriate corrections were applied to the calculated areas to compensate for such experimental variation as modulation broadening or insufficient scan width.[22]

P700 estimations were based on the value of $\Delta\epsilon \simeq 64$ mM^{-1} cm^{-1} at 703 nm recently reported by Hiyama and Ke.[23] It should be noted that their determination of $\Delta\epsilon$ was based on an optical measurement of a cytochrome coupling or reduced dye reaction and hence the $\Delta\epsilon$ is for a *one electron* equivalent change in the P700 chlorophyll. Recently Norris, *et al.*,[24] have postulated that in fact the unpaired electron is delocalized over *two* chlorophyll a molecules in $P700^+$. Since our results are based on a *one electron equivalent* change for both optical and esr measurements, the question of whether or not the electron is delocalized over one or two chlorophylls would not affect our results.

The results of our comparative quantitation experiments are illustrated in Table I. The ratio of spins to P700 for subchloroplast particles is approximately 1.1 ± 0.2 while for intact chloroplasts this ratio is 1.2 ± 0.3. It is therefore evident that since $P700^+$ and signal I are present in the same concentration (within experimental error) and have identical decay characteristics, these species must reflect the same molecular identity.

Table I. Comparison of Electron Spin Resonance and Optical Results

Sample	[Spins] × 10^6 M[a]	[P700] × 10^6 M	[Spins]/[P700]
TSF1	7.60 ± 0.60	7.69 ± 0.77	0.99 ± 0.18
TSF1	4.40 ± 0.40	4.00 ± 0.40	1.10 ± 0.22
D144	2.34 ± 0.27[b]	1.99 ± 0.20	1.17 ± 0.26
TSF1	12.20 ± 0.61	11.65 ± 0.60	1.05 ± 0.13
D144	10.40 ± 0.60	8.50 ± 0.85	1.23 ± 0.20
Chloroplasts	1.9 ± 0.3	1.6 ± 0.2	1.2 ± 0.3

[a] Power = 30 mW (modified cavity). Modulation amplitude 6.3 G. [b] Determined by rapid-scan procedure.

One is tempted to speculate why earlier chloroplast determinations indicated a nonequivalence of spins and P700 moieties. It must be emphasized that determinations of this type are inherently subject to manifold errors. Although Beinert and Kok[13] took numerous precautions during their quantitation investigations, the variability of their reported data indicates that not all experimental variables were controlled or recognized. It should be noted that their P700 determinations were made at room temperature whereas the esr measurements were usually made at low temperatures ($\sim -70°$). Sometimes they used extracted or subcellular preparations in lieu of intact cells and the P700 concentrations were consequently estimated on the basis of chlorophyll content. In addition, the value assumed for $\Delta\epsilon$ (*e.g.*, 80 mM^{-1} cm^{-1}) would underestimate the P700 content by approximately 25%.

Although the early attempts of Beinert and Kok[13] to clarify the relationships of P700 to signal I were unsuccessful, the foresight of these researchers has served as a stimulus and a challenge to those who have followed.

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