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EXPERIMENTAL DETERMINATION OF THE MOLAR DIFFERENTIAL EXTINCTION COEFFICIENT OF P700*

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SUMMARY

The molar differential extinction coefficients of P700 at 435 and 703 nm have been determined by using a directly coupled reaction in the dark involving the reduction of photooxidized P700 and the oxidation of reduced cytochromes.

From the coupled oxidation of mammalian cytochrome *c* by Anabaena high-P700 particles, the molar differential extinction coefficients of P700 at 435 and 703 nm are calculated to be $8.6 \cdot 10^4$ and $1.20 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$, respectively, and the ratio of the red to blue band heights is 1.4.

From the coupled oxidation of Euglena cytochrome-552 by Triton-fractionated pigment system 1 subchloroplast particles enriched in P700, the calculated molar differential extinction coefficients of P700 at 435 and 703 nm are very close to the values given above.

Using an absorbance decrease at 580 nm as a measure of the photoreduction of dichlorophenolindophenol by Triton-fractionated pigment system 1 subchloroplast particles enriched in P700 tends to yield a low extinction value because of other absorbance changes which occur at this wavelength and the non-reproducibility of the values obtained.

Comparisons are made between the extinction values of P700 and the corresponding extinction values of the bacteriochlorophyll reaction centers in photosynthetic bacteria.

INTRODUCTION

By measuring light-induced absorption changes in the far-red absorption region of a wide variety of photosynthetic organisms such as a blue-green alga (Nostoc), a red alga (Porphyra), green alga (Chlorella), as well as spinach chloroplasts, KOK[1] observed an ubiquitous absorption decrease near 700 nm which was reversible and recovered in the dark. Subsequently, KOK[2] suggested that the changes were caused by a chlorophyllous pigment acting as the final light trap in photosynthesis. By extracting 85 % of the bulk chlorophyll from chloroplasts, KOK[2] obtained a partially

Abbreviations: DCIP, dichlorophenolindophenol; PMS, phenazine methosulphate; DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

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purified pigment complex which retained the light-induced absorption change, and behaved as a one-electron oxidation–reduction system, with $E'_0 = +430$ mV. The major bands in the oxidized-*minus*-reduced or light-*minus*-dark difference spectrum were observed near 700 and 430 nm.

It was subsequently found that whenever pigment system 2 is inactivated (*e.g.* by ageing[3] or by adding 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)[4]), or when pigment system 2 is blocked from pigment system 1 (*e.g.* by sonication[5]), the light response of P700 can be isolated and observed[6]. In intact organisms, the P700 response is especially prominent in red[1] and blue-green algae[7,8], and a push-pull effect (reduction and oxidation, respectively) on P700 by the two pigment systems can be clearly demonstrated in the blue-green algae *Anacystis nidulans*[7] and *Plectonema boryanum*[8].

Recent use of detergents for fractionating chloroplasts has yielded photochemically active pigment system 1 particles enriched in P700 (refs. 9–11). These particles have provided a useful reaction system for studying photochemical reactions associated with pigment system 1 (ref. 11). More recently, detergent fractionation of chloroplasts from spinach and blue-green algae that are devoid of carotenoids, either by organic-solvent extraction[12,13] or by growth in the presence of diphenylamine[14], have yielded particles which are even further enriched in P700.

In spite of the knowledge accumulated on the properties of P700, one of the most important physical constants, namely, the molar extinction coefficient, has not yet been determined. This difficulty remains because pure preparations of P700 have not yet been achieved. It is obvious, however, that quantitative estimations of the amount of P700 present in natural pigment systems require an accurate value of the molar extinction coefficient. Brief mention has been made in the literature regarding determinations of the molar extinction coefficient of P700 through correlation with the amount of ferricyanide reduced[15] or dichlorophenolindophenol (DCIP) reduced[16]. The reported values are, respectively, $3.6 \cdot 10^4$ and $4.2 \cdot 10^4$ $M^{-1} \cdot cm^{-1}$.

In this paper we describe a procedure used to determine the differential molar extinction coefficient of P700, using a directly coupled reaction in the dark involving the reduction of photooxidized P700 and the oxidation of cytochromes whose extinction coefficients are well known.

MATERIALS AND METHODS

For the P700 preparations we have used the so-called high-P700 (HP700) particles isolated from Anabaena cells grown in the presence of diphenylamine[14] and the Triton subchloroplast particle, TSF-1 (formerly designated as PD-10) prepared from spinach[11]. The Anabaena particles contain negligible cytochrome *f*. Mammalian cytochrome *c* (Sigma) was used with Anabaena HP700 particles and Euglena cytochrome-552 was used with the TSF-1 particles. Detailed composition of the reaction mixture is indicated in the figure legends. The flash spectrophotometric technique has also been described earlier[17]. Depending on the magnitude of the light-induced signals, 1 to 32 flashes were used to achieve a desirable signal to noise ratio and to allow accurate reading of the data points from the kinetic traces. In all cases, flashes with 100 μsec halfwidth were used. Bandwidth of the excitation and measuring lights are also described in the figure legends.

RESULTS

Coupled oxidation of mammalian cytochrome c by Anabaena high-P700 particles

The high-P700 particles from Anabaena can oxidize mammalian ferrocytochrome *c* in the dark following an actinic flash; the cytochrome oxidation is directly coupled to the dark reduction of the photooxidized P700. Absorbance-change transients at 435 (P700) and 550 (cytochrome) elicited by 100 μsec flashes in the region of 650–750 nm are shown in the upper part of Fig. 1; the lower part shows the 703 (red band of P700) and 550 nm changes elicited by flashes in the region of 400–460 nm. The cytochrome *c* was reduced initially by catalytic hydrogenation before adding to the reaction mixture. Additional dithiothreitol (2,3-dihydroxy-1,4-dithiobutane) was added to maintain the cytochrome in the reduced state. Methyl viologen was added as the electron acceptor, since its presence was necessary to obtain reproducible results. It is possible that the presence of methyl viologen helps eliminate cyclic back reactions which involve the oxidized cytochrome *c*.

In the absence of cytochrome *c*, but with dithiothreitol present, the negative absorbance changes at 435 and 703 nm and the small absorbance increase at 550 nm decay very slowly, in a matter of several seconds (see top traces of Fig. 1). When

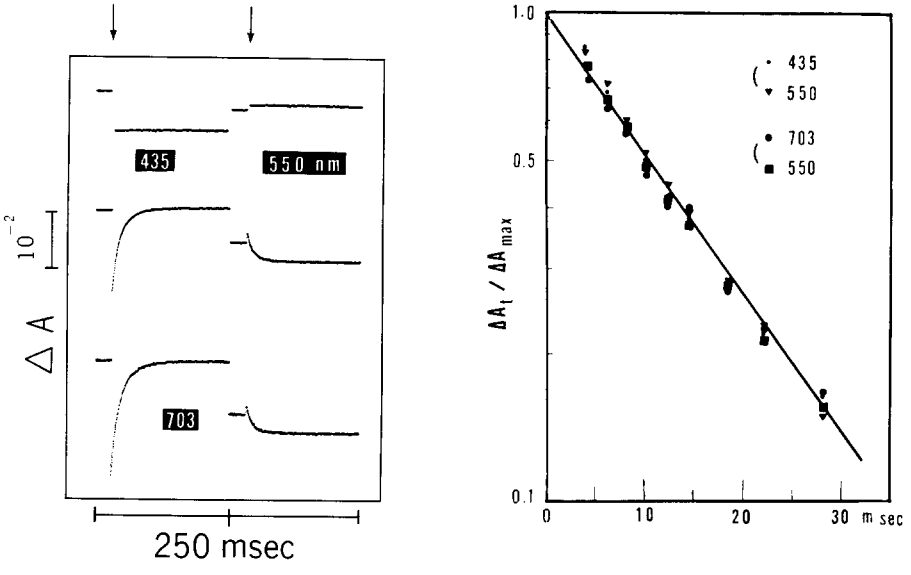


Fig. 1. Light-induced absorbance changes in Anabaena high-P700 preparation in the absence (top transients) and in the presence (the remaining four transients) of mammalian cytochrome *c*. Wavelength ranges of the excitation light: 650–750 nm for the 435 and 550 nm changes and 400–460 nm for the 703 and 550 nm changes. Arrow indicates the moment the 100 μsec flash was applied. The initial flat portion represents the baseline. Bandwidth of the measuring light, 1 nm. Chlorophyll, 10 μg/ml; cytochrome *c* (when present), 10 μM; dithiothreitol, 100 μM; methyl viologen, 50 μM. 0.01 M Tris at pH 7 was used for all experiments. Time scale, 125 msec total span for each trace.

Fig. 2. Kinetic plots of the absorbance changes at 435, 550 and 703 nm *vs.* time. The data points were read from separate recordings of the oscilloscope traces of Fig. 1. The recordings were made on an expanded scale to allow accurate estimate of the data points. Reaction conditions were the same as in Fig. 1.

TABLE I

RATIOS OF LIGHT-INDUCED ABSORBANCE CHANGES AND THE CALCULATED MOLAR DIFFERENTIAL EXTINCTION COEFFICIENTS OF P700

Reaction system *	$\frac{\Delta A_{435\ nm}}{\Delta A_{550\,(or\ 552)\ nm}}$	$\varepsilon_{(435\ nm)} \times 10^{-3}$ $(M^{-1} \cdot cm^{-1})$ **	$\frac{\Delta A_{703\ nm}}{\Delta A_{550\,(or\ 552)\ nm}}$	$\varepsilon_{(703\ nm)} \times 10^{-3}$ $(M^{-1} \cdot cm^{-1})$ **	$\frac{\varepsilon_{(703\ nm)}}{\varepsilon_{(435\ nm)}}$
Anabaena HP700 + mammalian cytochrome c^{2+} (a)	4.6 ± 0.2	86	6.4 ± 0.3	120	1.40
Spinach TSF-1 fraction + Euglena cytochrome-552 (b)	4.3	85	6.2	122	1.44

* Typical composition and experimental conditions for these reaction systems are described in Figs. 1 (a) and 3 (b), respectively. The Anabaena HP700 results are the average of 6 separate sets of experiments. Due to limited availability of crystalline Euglena cytochrome-552, only one experiment could be performed for system (b).

** In calculating the molar extinction coefficients at 435 and 703 nm for P700, the value of $18.7 \cdot 10^3\ M^{-1} \cdot cm^{-1}$ was used for mammalian cytochrome *c* at 550 nm[18] and $19.6 \cdot 10^3\ M^{-1} \cdot cm^{-1}$ was used for Euglena cytochrome-552 at 552 nm[19].

ferrocytochrome *c* was added, rapid decays with identical halftimes of 10 msec were observed at all three wavelengths. Increasing cytochrome concentration several fold only accelerated the decay rate slightly, with no effect on the signal magnitude. Also, the momentary kinetic correspondence was always maintained between the P700 and cytochrome signals. The close correspondence of the decay kinetics is further shown in Fig. 2, in which the data points were taken from separate recordings at a higher signal-to-noise ratio and replotted. Within experimental error, an exact kinetic correspondence is obtained for the two sets of transients excited by flashes of two different wavelengths.

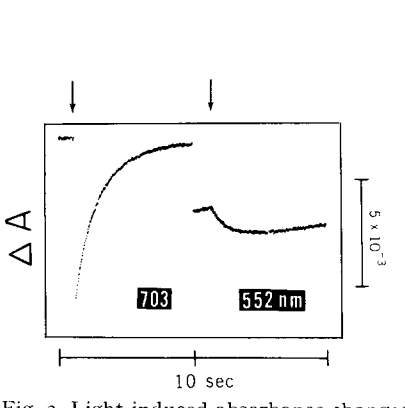
Since both P700 and cytochrome *c* are known to undergo one-electron redox reactions, and since the molar differential (reduced-*minus*-oxidized) extinction coefficient for cytochrome *c* at 550 nm is known with high precision[18], it is possible to relate the magnitudes of the absorbance changes of the two substances within the time span where good kinetic correspondence was maintained. For this purpose, only the net negative absorbance change of cytochrome *c* at 550 nm was taken, since the positive change occurred even when no cytochrome *c* was present. It will be shown later that this initial fast absorbance increase at 550 nm is associated with the photobleaching of P700, and the subsequent decrease was coupled to the oxidation of the cytochrome. The ratios of absorbance changes averaged from 6 sets of separate experiments are presented in Table I. By using the molar differential extinction coefficient of $1.87 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ for cytochrome *c*, as determined by MARGOLIASH AND FROHWIRT[18], the molar differential extinction coefficients of P700 at 435 and 703 nm were calculated to be $8.6 \cdot 10^4$ and $1.20 \cdot 10^5\ M^{-1} \cdot cm^{-1}$, respectively, and the ratio of red to blue band heights is 1.4.

Coupled oxidation of Euglena cytochrome-552 by Triton-fractionated pigment system 1 subchloroplast particles enriched in P700

The pigment system 1 particles, TSF-1, isolated from spinach, do not oxidize

mammalian cytochrome *c* but can oxidize cytochrome-552 from Euglena[11]. Since the differential extinction coefficient of cytochrome-552 from Euglena has been reported[19], we have also examined its oxidation by the photochemically oxidized P700 in the spinach subchloroplast particles. The reaction mixture consists of TSF-1 particles and Euglena cytochrome-552 at a relatively high concentration. The absorbance changes in the absence and in the presence of the cytochrome were similar to those observed with the Anabaena HP-700 particles, except that the rate of cytochrome oxidation was about 2 % as fast. However, the absorbance-change-transient profiles were very similar in the two cases. The absorbance-change transients at 703 and 552 nm, excited by 100 μsec flashes of 400–460 nm actinic light, are presented in Fig. 3, and the calculated molar differential extinction coefficients of P700 at 435 nm and 701 nm are included in Table I. Note that the extinction values for spinach P700 are in excellent agreement with those of the Anabaena P700.

In order to ascertain that only the net negative absorbance change at 550 nm should be taken into account in the correlation with the P700 absorbance changes, we have examined the absorbance changes in the Anabaena P700 particles in the absence and in the presence of different amounts of reduced phenazine methosulphate (PMS) (added to facilitate the rapid recovery of the P700 following the actinic flash. No absorbance changes due to PMS are observed at the wavelengths under consideration). The kinetic plots for the absorbance changes at 435, 703, and 550 nm at 0, 1.6, 3.2 and 16 μM PMS (in the presence of excess ascorbate) and one set of the transients at 16 μM PMS are presented in Fig. 4. From the kinetic correspondence of the absorbance changes, it is clear that the positive absorbance increase at 550 nm is a part of the changes occurring during the photooxidation of P700, and it decays



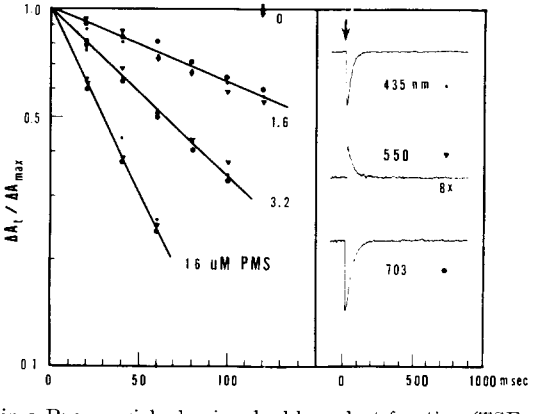


Fig. 3. Light-induced absorbance changes in a P700-enriched spinach chloroplast fraction (TSF-1) in the presence of Euglena cytochrome-552. Excitation-light wavelength range, 400–460 nm. Arrow indicates the moment the 100 μsec flash was applied. Measuring-light bandwidth, 1 nm. Total chlorophyll 15 μg/ml; cytochrome-552, 25 μM; dithiothreitol, 200 μM; methyl viologen, 50 μM. Time scale, 5 sec total span for each trace.

Fig. 4. Light-induced absorbance changes in Anabaena high-P700 preparation in the presence of different concentrations of reduced phenazine methosulfate. Left: kinetic plots of the absorbance changes *vs.* time at three PMS concentrations (0, 1.6, 3.2 and 16 μM). Right: actual absorbance-change transients at 435, 550 and 703 nm at 16 μM PMS. Experimental conditions similar to those in Figs. 1 and 3. Total chlorophyll, approx. 5 μg/ml; dithiothreitol, 100 μM; PMS (as indicated); methyl viologen, 50 μM. The 435 and 703 nm changes were induced by a single flash; that at 550 nm were averaged from 8 flashes.

rapidly and completely back to the baseline when an appropriate amount of an electron donor is present. Thus, the exact kinetic correspondence observed earlier in Figs. 1 and 3 for the 435, 550 (or 552) and 703 nm changes is also explained. Note that the ratio of the magnitudes of absorbance changes at 703 nm and 435 nm also agrees with those determined from the cytochrome-coupled reactions.

Photoreduction of DCIP by Triton-fractionated pigment system 1 subchloroplast particles enriched in P700

KOK[20] and KE[21] have reported previously that a rapid reduction of DCIP by pigment system 1 also takes place in addition to a slow and steady reduction of DCIP by pigment system 2 in spinach chloroplasts. The rapid-phase reduction by pigment system 1 was also confirmed by poisoning pigment system 2 by DCMU, by using Triton-fractionated subchloroplast particles, and by using far-red excitation light[21]. SCHLIEPHAKE *et al.*[16] recently reported the use of this reaction for a determination of the extinction coefficient of P700, the rationale being that the reduced primary electron acceptor (X) formed in the primary photochemical act of photosystem 1 would be the electron source for DCIP reduction, and that the amounts of reduced primary electron acceptor and the photooxidized P700 should be equivalent. SCHLIEPHAKE *et al.*[16] stated that "using the change of the extinction coefficient of DCIP at its reduction as determined by Punnett, the change of the extinction coefficient of chlorophyll a_1 (P700) at its oxidation has been determined as $4.2 \cdot 10^4$ l·mole^{-1}·cm^{-1}".

Because of the discrepancy in the numerical values obtained by us using the cytochrome-coupled reaction with P700-enriched particles and those reported from WITT's laboratory[15,16], we have reexamined the properties of the DCIP reduction with various P700-enriched particles at a higher time resolution. The particles were first treated with a low concentration of ascorbate (10 μM for approx. 10 μg chlorophyll per ml) to bring the P700 to the maximum reduced state, and then DCIP was added to 100 μM. We have chosen 580 nm for following DCIP reduction, as the absorbance change associated with P700 reaction is minimal at this wavelength. Light-induced absorbance-change transients in such a reaction mixture at 435, 703 and 580 nm are presented in Fig. 5, and detailed experimental conditions are described in the figure legend. It can be seen from Fig. 5 that P700 undergoes rapid photooxidation (represented by the 435 and 703 nm transients) and decays slowly since the amount of reduced DCIP would be small under these conditions. At 580 nm the rise of the absorbance change consists of a rapid portion and a slower portion ($t_{1/2}$ approx. 2–4 msec; see the magnified 580 nm transient immediately next to the 703 nm transient in Fig. 5). Taking the total magnitude of the absorbance change at 580 nm as that due to DCIP reduction and taking into account that DCIP reduction is a two-electron reaction, the extinction values for P700 measured with the Triton-fractionated subchloroplast particles, spinach high-P700 particles[12] and the Anabaena high-P700 particles[14] were $4.6 \cdot 10^4$–$6.6 \cdot 10^4$ M^{-1}·cm^{-1} for 435 nm and $5.0 \cdot 10^4$–$7.3 \cdot 10^4$ M^{-1}·cm^{-1} for 703 nm (18.5 (= 20 × 0.925) was used as the millimolar extinction coefficient for DCIP at 580 nm at pH 7).

Although earlier experiments[21] on rapid reduction of DCIP by pigment system 1 was considered with reference to the slow and steady reduction by pigment system 2, the results obtained here at a higher time resolution suggest that the initial rapid portion is associated with the P700 reaction and that only the slower portion with

a $t_{1/2}$ approx. 2–4 msec is due to DCIP reduction. KOK *et al.*[20] previously reported a risetime of 1.6 msec at 2.5 μM DCIP and 40 μsec for 0.1 mM DCIP, respectively. Since the extinction coefficient determinations from Witt's laboratory were made with 10 msec flashes (as specified in ref. 16), any variation in the rise kinetics at the DCIP wavelength could not have been resolved.

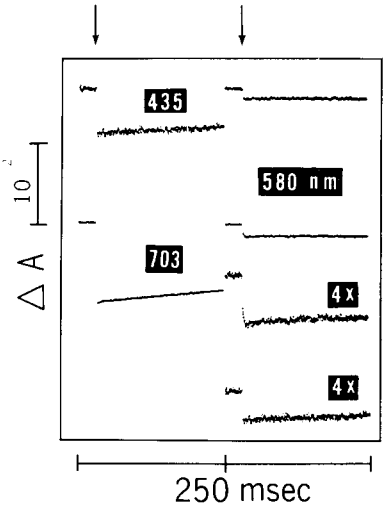


Fig. 5. Light-induced absorbance changes in a spinach chloroplast fraction (TSF-1) enriched in P700 at 435 nm, 580 nm and 703 nm in the presence of oxidized dichlorophenol indophenol. Experimental conditions and other designations the same as in Fig. 1. Total chlorophyll, approx. 10 μg/ml; ascorbate was added first to 10 μM followed by DCIP to 100 μM. Traces were obtained by averaging 32 flashes. One of the 580 nm transient is presented at 4-fold higher sensitivity in order to show the slower portion. Immediately below this expanded transient is that obtained when methyl viologen was present (also at a 4× expanded sensitivity).

Although it is in principle possible to resolve this question by measuring the difference spectrum for the slower-rising changes over the DCIP absorption range, the broad absorption spectrum of DCIP makes such a determination tedious and unfruitful. Instead, we have performed a simpler experiment to demonstrate that only the slower decrease at 580 nm originates from DCIP reduction. The experiment involves the addition of both DCIP and methyl viologen (both at 100 μM) to the reaction mixture. When methyl viologen was present, the slower-rising portion was completely absent, whereas the rapid-rising portion was quantitatively retained. These results indicate that methyl viologen can presumably more favorably compete for the electron from the primary electron acceptor and thus the slower-rising portion of the absorbance change at 580 nm due to DCIP reduction could not be seen.

When the absorbance change specifically due to DCIP reduction is properly taken into account by correcting for the fast-phase change, a higher extinction value for P700 consistent with those reported in Table I is obtained. But in view of the lack of a good stoichiometry and exclusiveness between the primary electron acceptor and DCIP (in the absence of methyl viologen, oxygen could also be a potential recipient of the electrons), the DCIP reduction reaction does not appear to be a reliable system for estimating the extinction coefficient of P700.

DISCUSSION

The directly coupled reaction between reaction-center bacteriochlorophyll and endogenous *c*-type cytochrome has only recently been demonstrated by rapid kinetic spectrophotometry in chromatophores[22] and subchromatophore fragments[23,24] of the photosynthetic bacterium, *Chromatium*. More recently, a directly coupled reaction between the reaction-center bacteriochlorophyll, P870, isolated from a mutant strain R-26 of *Rhodopseudomonas spheroides* and added mammalian cytochrome *c*, with an electron-transfer time approaching that of intact cells, was also observed[25].

Direct coupling between the reaction-center chlorophyll, P700, and endogenous cytochromes in either green plants or algae has not yet been definitively established (*cf.* refs. 26–28). However, Triton-fractionated subchloroplast particles (TSF-1) enriched in P700 are capable of oxidizing an exogenously added cytochrome-552 from Euglena[11]. The direct coupling in this reaction has now been demonstrated in the present study.

Oxidation of mammalian cytochrome *c* by lamellar fragments from spinach requires a protein factor[29], which was subsequently identified as plastocyanin[30,31]. On the other hand, FUJITA *et al.*[32,33] found that lamellar fragments from the blue-green algae *Anabaena cylindrica* and *Anacystia nidulans* can oxidize mammalian cytochrome *c* without plastocyanin. Again, the data reported above show conclusively a direct coupling between mammalian cytochrome *c* and P700 in the Anabaena HP700 particles.

The good interaction stoichiometry resulting from the direct coupling between P700 and a *c*-type cytochrome furnishes a satisfactory means by which the molar differential extinction coefficient of P700 can be estimated. The validity of this approach is shown by the *in vitro* reaction mentioned above which involves the reaction-center complex isolated from a mutant strain of *R. spheroides* and mammalian cytochrome *c*: a 1:1 interaction stoichiometry was confirmed by assuming the extinction coefficient values reported in the literature for cytochrome *c* and P870 (ref. 25). In the present case, the estimation of the extinction values of P700 by utilizing the cytochrome-coupled reaction has one further advantage: the fact that the wavelength of the Soret-band maximum of P700 at 435 nm is also the isosbestic point in the oxidized-*minus*-reduced difference spectrum of cytochrome *c*[18], though coincidental, has greatly facilitated these measurements. The results presented in Table I show a good reproducibility for the coupled reaction induced by exciting the two major absorption bands of chlorophyll, and there was good agreement in the extinction values calculated for P700 in the spinach subchloroplast particles and Anabaena HP700 particles.

It is also worth noting that although the Triton-fractionated spinach subchloroplast particles (TSF-1) can oxidize Euglena cytochrome-552, we have not been able to observe the same reaction with the spinach HP700 particles that were prepared from chloroplasts extracted with organic solvents to remove carotenoids prior to Triton fractionation[12]. Presumably, organic-solvent treatment has altered or removed some structural factors that are necessary for the direct interaction with the Euglena cytochrome.

The P700 extinction coefficient estimated here from the cytochrome-coupled reaction is the differential extinction value. The actual extinction values at these

absorption peaks could either be the same or greater than those reported. It is also of interest to note that, unlike chlorophyll in organic solutions or the bulk chlorophyll *in vivo*, where absorption in the blue band is greater than that of the red band, the red band in P700 is more intense. The red-to-blue ratio of greater than unity has been observed by many workers[2,4,8,31], but the theoretical significance of the relative band heights has not yet been elucidated. The large shift of the major red absorption band in P700 indicates that it represents chlorophyll situated in a special environment, which endows it with the ability to serve as the first link between photochemistry and chemistry. Based on previous studies on artificial aggregates of chlorophyll *a*[35,36], certain inferences have been made as to the nature of the chlorophyll in P700. However, the exact nature of the environment which leads to the unusual spectral properties of P700 remains unknown, and it should be the focus of future work in this area.

The counterparts of P700 in photosynthetic bacteria, namely, P870 in *R. spheroides* or P890 in *Chromatium* or *Rhodospirillum rubrum*, are bacteriochlorophyll molecules which are situated in a special environment and are bestowed with photochemical activity. The studies of the reaction-center bacteriochlorophyll has been facilitated by the fact that the bulk light-harvesting bacteriochlorophyll can be preferentially destroyed by oxidation[37] or extracted with detergents[33] without harming either the reaction-center bacteriochlorophyll, P870, or P800. By selectively oxidizing the chromatophores (K_2IrCl_6 bleaches the bulk bacteriochlorophyll; K_2IrCl_6 *plus* $K_3Fe(CN)_6$ bleach P870 in addition) and by extracting the bacteriochlorophyll in such chromatophores with an acetone–methanol mixture, CLAYTON[37] concluded that the pigments in the reaction-center complex are in a molecular ratio of 1 P870:2 P800. Based on this assumption, the molar extinction coefficients of bacteriochlorophyll in P870 and P800 were calculated to be $1.13 \cdot 10^5$ and $1.36 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$, respectively. Thus, the magnitude of the extinction values estimated for P700 and P870 show a comparable enhancement relative to those of isolated chlorophylls in solution.

Thus far, the red-band extinction coefficients of chlorophyll in solution or of the bulk chlorophyll *in vivo* have been used to estimate P700 content in pigment complexes. If the extinction values reported here are considered, most of the literature values given for the P700 concentrations would have to be revised downward. In the early literature on ESR studies, BEINERT AND KOK[39] had assumed the red-band molar extinction of P700 to be $8 \cdot 10^4$–$1 \cdot 10^5$ $M^{-1} \cdot cm^{-1}$ and estimated the ratio of spins per P700 to be about 2 for a wide variety of photosynthetic materials studied. More recent studies, however, showed a spin-to-P700 ratio closer to unity[40]. In view of the availability of these high-P700 particles together with the newly determined extinction values, a reexamination of the spin-to-P700 relationship appears warranted.

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