(70 μg) treated in this way also gave satisfactory results.

*Other Conditions Tested.* The inclusion of either tRNA or poly(adenylic acid) alone (Wagner *et al.*, 1967) without the steps described above gave results which were less satisfactory than those of Figure 3. In another experiment, samples of rRNA in $Me_2SO$ were diluted with either two volumes (Figure 4a) or four volumes (Figure 4b) of NETS buffer and layered directly onto sucrose gradients (Lindberg and Darnell, 1970). Although there was no difficulty in layering the samples, the sedimentation profiles were very broad, compared to methods in which ethanol precipitation of the RNA was used.

A different problem was encountered in attempting to dissolve ethanol-precipitated RNA directly in aqueous $Me_2SO$ solvents. The solvents used are indicated in the legend to Figure 5. Relatively small changes in the composition of the solvent had a pronounced effect on the results. It is clear that in Figure 5a,b the RNA had not dissolved adequately, since the $Me_2SO$-induced denaturation was not complete. Only in Figure 5c are the results comparable to those of Figure 3.

The present experiments were designed to provide practical information concerning the use of $Me_2SO$ as a denaturing agent for rRNA. One of the major problems encountered was aggregation which seems to be due to a tendency of denatured RNA molecules to form base-paired regions (intermolecular as well as intramolecular) when the denaturing condition is removed. This phenomenon resembles aggregation of rRNA which has been induced by heat treatment (Stanley and Bock, 1965; Marcot-Queiroz and Monier, 1965; Wagner *et al.*, 1967; Lovett and Leaver, 1969). Our results suggest that intermolecular bond formation can be minimized by dilution of the RNA and the use of low ionic strength solutions as described. Another potential complication was that precipitated RNA did not dissolve readily in some solvents containing high concentrations of $Me_2SO$. This could be misleading since denaturation of the RNA would likely be incomplete.

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# Exciton Interaction in the Photosystem I Reaction Center from Spinach Chloroplasts. Absorption and Circular Dichroism Difference Spectra†

Kenneth D. Philipson, Vicki L. Sato, and Kenneth Sauer*

ABSTRACT: Preparations enriched 10-fold in the photosystem I reaction center chlorophyll, P700, have been studied by observing the changes which occur in both the absorption and circular dichroism upon illumination. The results suggest that there are at least two chlorophyll a molecules within the reaction center and that a significant exciton interaction exists among them. Upon photoactivation, one molecule within the array becomes oxidized, leading to the loss of exciton interaction. It is proposed that these intimate associations among the chlorophyll molecules are an integral part of the operation of the photosystem I reaction center.

The chlorophyll molecules found within the chloroplasts of higher photosynthetic organisms are of two distinct functional types. The large majority of pigment molecules are antenna, or bulk, chlorophylls, and a smaller fraction is contained in what is known as the reaction center. The function of the antenna chlorophylls is to absorb incident light

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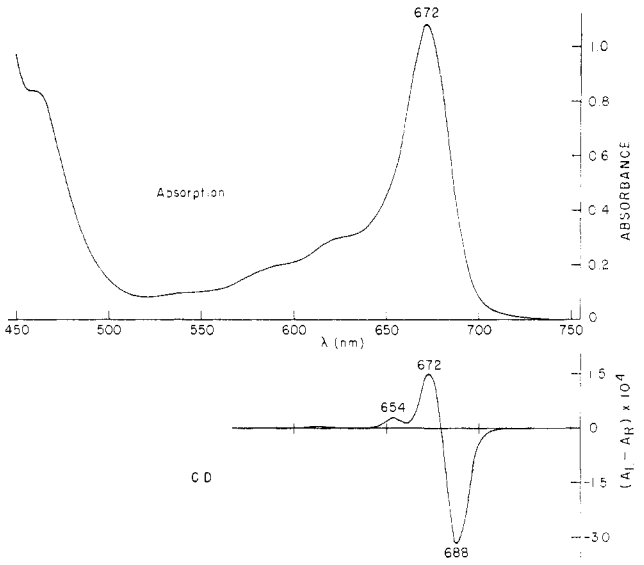


FIGURE 1: Absorption and circular dichroism spectra of reduced HP700 particles; phosphate buffer (pH 7.4, 0.01 M KCl); 1-cm path length and 25°; chlorophyll: P700 is 60:1.

photons and to transfer this energy, in the form of electronic excitation, to the reaction center chlorophylls where the primary photoproducts of photosynthesis are formed. Both types of chlorophylls are embedded in the lipoprotein matrix of the thylakoid membrane.

A light-induced reversible absorbance change at 700 nm in photosynthetic organisms, initially reported by Kok in 1956, has been associated with the oxidation of a specific chlorophyll a molecule (P700) within the reaction center of photosystem I (Kok, 1956). The concentration of P700 within the chloroplast, however, is low (*ca.* 1 400–600 chlorophylls/P700) and, despite much effort, little is known of the molecular architecture of this reaction center.

The use of organic solvents and solubilizing detergents to extract the bulk chlorophylls has allowed the preparation of fractions enriched in P700, greatly facilitating the examination of this photoactive reaction center pigment. In contrast, however, to bacterial systems from which highly purified reaction centers have been obtained (Clayton, 1963), no completely purified preparation of green plant reaction centers has yet been achieved. Recent preparations contain chlorophyll to P700 ratios of 15–30:1 (Sane and Park, 1970; Ogawa and Vernon, 1969; Yamamoto and Vernon, 1969). This has presented a serious obstacle to the characterization of P700 within the reaction center, for the presence of large amounts of bulk chlorophyll often presents difficulties in the interpretation of optical data.

Studies of purified bacterial reaction centers have shown that circular dichroism (CD) spectroscopy can provide new insights into the organization of pigments within the reaction center (Sauer *et al.*, 1968; K. D. Philipson and K. Sauer, submitted). These studies on the bacteriochlorophylls of purple bacteria suggested a model in which the reaction center consists of at least three strongly interacting bacteriochlorophyll molecules, one of which is oxidized by light.

In this paper the results of some similar studies with CD and absorption spectroscopy on a highly enriched preparation of green plant reaction centers are considered. The spectral changes observed upon illumination of these P700 particles strongly support a model for the photosystem I reaction center which involves exciton interaction between at least two molecules of chlorophyll a.

## Materials and Methods

Preparations of enriched P700 particles (HP700), fractionated from spinach by treatment with organic solvents, Triton X-100, and sucrose density centrifugation were described by Yamamoto and Vernon (1969). We found, however, that the use of 0.025% Triton X-100 in tricine buffer for the initial detergent treatment of the hexane–acetone-extracted chloroplasts was sufficient to release residual accessory pigments and bulk chlorophyll. The 0.25% Triton concentration reported by Yamamoto and Vernon frequently resulted in low yields of the HP700 fraction, presumably due to the harsher action of the higher detergent concentration. Purified preparations were stored in darkness at −20°, under which conditions they were stable for 2 weeks. Samples obtained had chlorphyll:P700 ratios of 37–60:1.

Absorption spectra were measured on a Cary 14R recording spectrophotometer fitted with a 150-W tungsten lamp for side illumination of the sample cuvet. For the light-minus-dark spectra, the photomultiplier was shielded with a Corning 2-58 filter, and the sample was illuminated with actinic light filtered through a Baird Atomic 436-nm interference filter and a Corning 5-60 filter. The resultant light had an intensity of $5 \times 10^4$ ergs $cm^{-2}$ $sec^{-1}$. Samples were chemically reduced with an excess of sodium ascorbate added in the solid form.

The chlorophyll to P700 ratio was determined by directly comparing the absorbance at 672 nm to the magnitude of the light-induced absorbance change at 697 nm.

CD spectra were recorded on a Durrum-Jasco J-20 CD spectrometer, also adapted for side illumination. The same filter combinations and light intensity cited for the Cary spectrophotometer were used here. The CD spectra are given in terms of $A_L - A_R$, where $A_L$ and $A_R$ equal the absorbance for left and right circularly polarized light, respectively.

Because it was found that continual illumination of a P700-containing sample for the duration of a CD spectral scan resulted in some irreversible bleaching of the sample, the light-minus-dark difference CD spectra were recorded in the following manner. Each ascorbate-reduced sample was allowed to equilibrate in the dark for several minutes, after which the dark CD signal at a particular wavelength was recorded. Actinic illumination was then begun, and the change induced in the CD signal of the sample monitored. The illumination period was approximately 2 min. Following illumination, each sample was allowed to recover in the dark, while the return of the CD signal to the dark level was recorded. This procedure was repeated at intervals of a few nanometers in order to determine the light-minus-dark difference CD spectrum.

Because of the small signal size [$\Delta(A_L - A_R) \cong 2.5 \times 10^{-5}$], special care was taken to exclude the possibility of signal artifacts created by the illumination procedure. Samples known to possess no photoactivity were subjected repeatedly to the dark-light-dark sequence described above, with no indication of CD changes induced by the procedure.

## Results

*Absorption and CD of Reduced HP700.* The absorption spectrum of an HP700 preparation which has been reduced with ascorbate is given in Figure 1. The peak of the absorption is at 672 nm, as compared to the 678 nm normally seen in in-

tact chloroplasts. This shift to shorter wavelengths in the HP-700 fraction is consistent with that reported by Yamamoto and Vernon (1969), although they found the peak at 676 nm. There is also a rather large chlorophyll b absorption shoulder at 645 nm, indicating a higher concentration of this pigment in our preparation than has been previously reported (Yamamoto and Vernon, 1969). The absence of any absorption around 500 nm, however, does indicate the removal of carotenoids from this fraction.

It should also be noted that the detergent treatment results in an HP700 preparation which exhibits very little turbidity.

The circular dichroism spectrum of an ascorbate-reduced HP700 preparation is shown in Figure 1, and three bands are apparent in the long-wavelength region. These components are at 688 nm (−), 672 nm (+), and 654 nm (+), where (+) and (−) indicate the signs of the CD signal. Although the CD spectrum does vary from one preparation of HP700 to another (as does the ratio of P700 to total chlorophyll), these basic features are retained.

*Light-Minus-Dark Absorption and CD.* The light-minus-dark absorption and CD spectra are both given in Figure 2. The absorption difference spectrum reveals two bands, a major decrease at 697.5 nm, and a smaller change at 680 nm. Both components are light-induced absorbance changes which are reversible in the dark. Although there is some variability in the size of the 680-nm feature, as is also noted by other investigators (Vernon *et al.*, 1969), the reversibility of the absorbance change strongly suggests that the peak does not represent nonspecific bleaching of bulk chlorophyll. It is significant that the asymmetry in the long-wavelength component of the spectrum is not normally observed for this chlorophyll absorption band.

The shape of the absorption difference spectrum given here is consistent with that reported for P700 by several other laboratories working on different types of preparations. The shape of the difference spectrum (one major band near 700 nm, another minor band near 680 nm) appears to be the same in preparations ranging in purity from untreated chloroplast fragments (Kok and Hoch, 1961) to the enriched HP700 particles (Ogawa and Vernon, 1969; Yamamoto and Vernon, 1969), although there is a shift of the major peak to shorter wavelengths (703 nm in chloroplast fragments, 697.5 nm in HP700) in fractionated preparations.

The light-minus-dark CD spectrum of the HP700 particles (Figure 2) exhibits two components at 696.5 nm (+) and 688 nm (−) of approximately equal areas but of opposite sign. Like the absorbance changes, these changes in the circular dichroism upon illumination are also reversed in the ensuing dark period. We observed similar changes in hexane–acetone-extracted chloroplasts which had not yet been subjected to detergent. Thus, this effect is not an artifact of the Triton X-100 treatment. Attempts to observe the effect in unextracted chloroplast fragments, with low P700 concentration, were limited by instrumental sensitivity. The long-wavelength positive CD change could be seen, but excessive noise, due to rising sample absorbance, made the accompanying negative change unobservable.

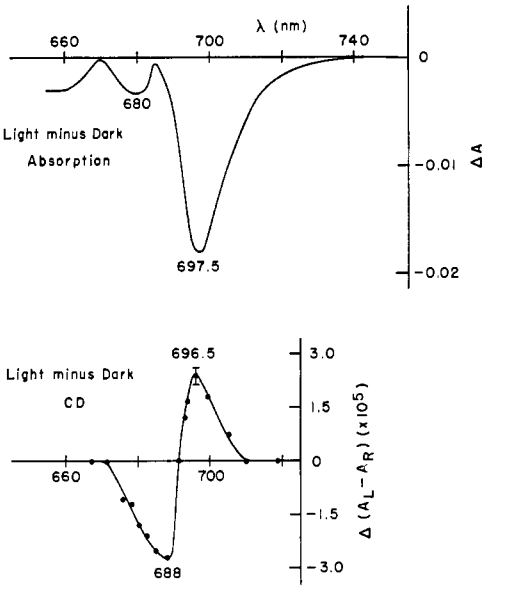


FIGURE 2: Absorption and circular dichroism difference spectra of HP700 particles; $A_{672}$ = 1.08; phosphate buffer (pH 7.5, 0.01 M KCl); 1-cm path length and 25°; chlorophyll:P700 is 60:1; see text for details.

## Discussion

Several laboratories have been successful in preparing highly enriched fractions of P700 which range in purity from 1 P700/80 chlorophyll molecules (Kok, 1961) to 1 P700/15 chlorophyll molecules (Sane and Park, 1970). Optical studies on the P700 of both chloroplast fragments (Kok and Hoch, 1961) and the enriched preparations are consistent in revealing the light-minus-dark difference spectrum with the shape shown in Figure 2. On the basis of the two bands (680 and 697.5 nm) evident in this difference spectrum, several workers (Murata and Takamiya, 1969; Döring *et al.*, 1968; Vernon *et al.*, 1969) have sugggested a model for the photosystem I reaction center which involves at least two molecules. Characterization of the oxidation–reduction properties of the absorbance change at 680 nm (Murata and Takamiya, 1969), revealed extensive similarities to those found at 700 nm. These two absorbance changes exhibited very similar kinetic behavior (Döring *et al.*, 1968). From these observations it has been suggested that the absorbance change at 680 nm is another expression of that seen at 700 nm.

Using the technique of difference circular dichroism spectroscopy, we have obtained results which imply that at least two chlorophyll a molecules are involved in the photosystem I reaction center; these molecules are apparently coupled by an exciton interaction (Kasha *et al.*, 1965). Under the conditions of exciton coupling, the electronic interaction among the chromophores is so strong that photon absorption results in the collective excitation of the array of reaction center chlorophyll molecules. Thus, it is not possible to excite an individual chlorophyll molecule within the array. A splitting of energy levels occurs as a result of this interaction, with the number of new energy levels (*i.e.*, absorption bands) equalling the number of molecules involved. The signs and rotational strengths of the CD band components resulting from exciton interaction are determined by the specific geometric arrangement of the interacting molecules with respect to one another, rather than by the inherent asymmetry of the individual molecules. The exciton contribution to the rotational strength is conservative (Tinoco, 1963), meaning that its contribution to the rotational strength should sum to zero over the exciton band. Thus, it is characteristic of exciton interactions for the different components within the one electronic band to have both positive and negative signs.

The presence of two components of approximately equal

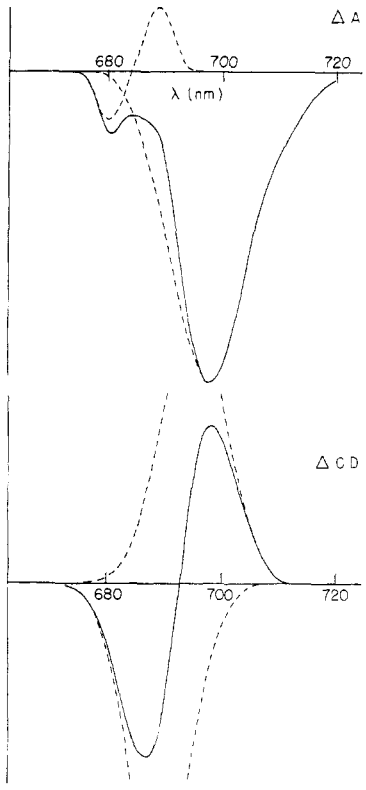


FIGURE 3: Resolution into components (- - - -) of curves (——) which are representative of the experimental absorption and CD difference spectra for HP700 particles. The components in the $\Delta A$ spectrum are at 683 (−), 686 (+), and 698 (−) nm; the latter has a half-width of 16.6 nm. For the $\Delta CD$ spectrum the components are centered at 689 (−) and 695 (+) nm. Both components have half-widths of 13.3 nm. The experimental results could not be resolved well into gaussian components, partly due to scatter in the data points; thus, the sums of these components (——) are not quantitative fits of the actual data. See text for details.

area but opposite sign in the light-minus-dark CD spectrum (Figure 2) strongly implies that an exciton interaction exists among the pigment molecules of the reaction center in the reduced state. Upon illumination, the oxidation of a specific chlorophyll a within the reaction center results in the loss of resonant coupling; the exciton states no longer exist. Thus, the conservative CD, due to exciton interaction in the reduced reaction center, must disappear upon light oxidation. The loss of this exciton contribution will be reflected in a conservative light-minus-dark CD spectrum.

The magnitude of the circular dichroism change [$\Delta(A_L - A_R) \cong 2.5 \times 10^{-5}$] which is seen in the HP700 particles is appreciable. Because the spectrum shown in Figure 2 was obtained from a preparation containing only 1 P700/60 chlorophylls, the fraction of chromophores in the sample which actually contribute to the signal is quite small. In addition, the magnitude of the difference CD signal is further reduced by the overlap of components of opposite sign. As can be seen from the curve resolution shown in Figure 3, this results in cancellation of a major part of the CD components. A CD change of this magnitude is consistent with a loss of an exciton contribution upon light oxidation.

We wish to emphasize that the foregoing discussion is based upon an analysis of a CD difference spectrum, in which advantage is taken of the reversible light-induced properties inherent in a photosynthetic reaction center. The circular dichroism of the reduced HP700 particles (Figure 1) provides little information about the reaction center itself; it is primarily an expression of the large number of bulk chlorophylls present in the preparation. It is also important to note that the long-wavelength $Q_y$ transition of chlorophyll a is electronically allowed and nondegenerate. This excludes the possibility of multiple CD components arising from one molecule; there is at least one molecule present for each component observed.

The light-induced absorbance changes exhibited by the HP700 particles are consistent with the existence of an exciton interaction which is lost upon oxidation of one chlorophyll molecule. Two negative components (680 and 697.5 nm) are seen in the absorption difference spectrum (Figure 2), the larger of which exhibits a marked asymmetry with the steep portion on the short-wavelength side. These spectral properties can be accounted for by the light-induced disappearance of the two observable exciton components and the associated appearance of a new absorption band due to a remaining, noninteracting chlorophyll. The bleaching of the exciton components is responsible for both negative changes. It is suggested by the skewed shape of the change at 697.5 nm that the absorption band of the noninteracting chlorophyll overlaps, and is therefore cancelled by, the larger negative components. A diagrammatic representation is given in Figure 3 (upper curves). The inflexed curve centered at 684.5 nm represents the band shift to longer wavelengths that has been previously reported to accompany P700 photooxidation (Vernon *et al.*, 1969). We interpret this feature to represent the simultaneous disappearance of a short-wavelength exciton component of P700, located at approximately 683 nm, and the appearance of a band at approximately 686 nm owing to the remaining unoxidized chlorophyll molecule in $P700^+$. This process can be summarized by

$$P700\ (Chl\text{–}Chl)_{683,697} \xrightarrow{h\nu} P700^+\ (Chl^+\text{–}Chl)_{686}$$

The apparent bandwidths (5.5 nm) associated with the shift centered at 684.5 nm seem anomalously narrow when compared to the 16.5-nm bandwidth of the trough at 697.5 nm. This confirms the proposal that a rather small difference (2–3 nm) exists between the two components involved in the "band shift." This apparent narrowing associated with the canceling of overlapping features is also evident in the analysis of the CD spectrum (Figure 3, lower curve).

Although the exciton model is in agreement with the suggestion of other investigators (Murata and Takamiya, 1969; Döring *et al.*, 1968; Vernon *et al.*, 1969) that at least two chlorophyll molecules are involved in the reaction center, it differs significantly in several respects. Unlike these previous models, which have not specified the nature of the relationship among the associated chlorophylls, our proposal involves strong electronic coupling of the chlorophylls within the reaction center. Thus, both the 680- and 700-nm absorbance changes must be considered characteristic of the aggregate of chromophores; the 680-nm change cannot be assigned to a separate pigment molecule which is only loosely associated with an oxidizable chlorophyll. Studies of the *in vivo* and *in vitro* electron paramagnetic resonance line widths have been interpreted in terms of strongly coupled chlorophyll molecules in the reaction centers of both higher plants (Norris *et al.*, 1971) and bacteria (McElroy *et al.*, 1972). The optical results presented in this paper are consistent with and complementary to these proposals.

Although the spectroscopic evidence presented here does not exclude the possibility that conformational changes of

reaction center proteins may cause the observed optical effects, we feel that the size and shape of the difference CD spectrum are most simply explained by the presence of exciton interaction.

The model of a photosystem I reaction center containing two chlorophylls should be regarded as a simplest hypothesis. Because of the presence of a large amount of antenna chlorophyll, we do not know the absorption and CD properties of either the reduced or the oxidized reaction center pigments. This is by contrast with the bacterial reaction center of *Rhodospirillum spheroides* (Sauer *et al.*, 1968), where the CD spectrum of the reduced form provides strong evidence for the participation of at least three bacteriochlorophyll molecules. For some bacterial reaction centers the light-minus-dark difference CD spectra (K. D. Philipson and K. Sauer, in preparation) have rather similar shapes to that observed for the HP700 preparation, except that the signs of the long- and short-wavelength components are reversed. Thus, while it is possible that the HP700 preparation reaction centers contain three instead of two participating chlorophyll molecules, the sign reversal demonstrates that the geometries of the bacterial and higher plant reaction centers are substantially different from one another.

Because of its sensitivity to exciton interactions, CD has proved to be particularly useful in the study of certain photosynthetic systems (Philipson and Sauer, 1972). Moreover, the technique of light-minus-dark CD offers unique advantages for examining photoactive components, even in preparations dilute in these components. Other studies (Sauer *et al.*, 1968; K. D. Philipson and K. Sauer, in preparation) on the absorbance and CD properties of purified reaction centers from photosynthetic bacteria imply the presence of exciton interactions within these systems. Thus, it appears that exciton interactions may play a fundamental role in the operation of both bacterial and green plant reaction centers.

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# Thermodynamic and Kinetic Studies of the Interconversion of Linear and Circular $\lambda b_2 b_5 c$ Deoxyribonucleic Acid in the Presence of Purine and Native Ribonuclease A†

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ABSTRACT: The melting temperatures and transition widths of the cohesive ends of $\lambda b_2 b_5 c$ DNA have been measured in the presence of varying concentrations of two helix-destabilizing ligands, purine and native ribonuclease A. The depression of the melting point in the presence of purine is in agreement with a model where purine binds only single-stranded DNA with one purine per nucleotide. The enthalpy of the cyclization equilibrium is unaffected by the presence of purine. The depression of the melting point in the presence of ribonuclease A is in agreement with a model of greater binding to denatured than native DNA with one protein molecule bound per cohesive end. The enthalpy of binding of ribonuclease A to denatured DNA is about −25 kcal/mole and to native DNA is about −13 kcal/mole. The rate of cyclization of $\lambda b_2 b_5 c$ DNA was measured in the presence of purine and ribonuclease A. In the presence of purine, the rate is reduced with no change in the enthalpy of activation. In the presence of ribonuclease A, no changes in cyclization rates were observed.

Ligands which bind to DNA may be classified as stabilizers or destabilizers. Stabilizers increase the stability of native DNA relative to denatured DNA, thus raising the melting temperature. Many positively charged planar dyes and positively charged proteins, as histones, are known to be stabi-

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