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ANTENNA FUNCTION OF A CHLOROPHYLL *a/b* PROTEIN COMPLEX OF PHOTOSYSTEM I

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The functional role of a chlorophyll *a/b* complex associated with Photosystem I (PS I) has been studied. The rate constant for P-700 photooxidation, $K_{P\text{-}700}$, which under light-limiting conditions is directly proportional to the size of the functional light-harvesting antenna, has been measured in two PS I preparations, one of which contains the chlorophyll *a/b* complex and the other lacking the complex. $K_{P\text{-}700}$ for the former preparation is half of that of the preparation which has the chlorophyll *a/b* complex present. This difference reflects a decrease in the functional light-harvesting antenna in the PS I complex devoid of the chlorophyll *a/b* complex. Experiments involving reconstitution of the chlorophyll *a/b* complex with the antenna-depleted PS I preparation indicate a substantial recovery of the $K_{P\text{-}700}$ rate. These results demonstrate that the chlorophyll *a/b* complex functions as a light-harvesting antenna in PS I.

Until recently the light-harvesting chlorophyll-protein complex (LHCP) of Photosystem (PS) II, which contains approximately equivalent amounts of chlorophyll *a* and *b*, appeared to be the only chlorophyll-*b*-containing protein in chloroplasts of green algae and higher plants [1,2]. However, the presence of a chlorophyll *a/b* protein complex associated with PS I in various photosynthetic organisms has recently been documented. A PS-I-associated complex, denoted CP_0, has been identified in *Chlamydomonas* [3,4], and another complex (LHCP I) in spinach and peas [5–8]. Our previous work [6,7], as well as those of others [5,8], has suggested that the PS I chlorophyll *a/b* complex is distinct from LHCP II, and specific antibodies have shown that LHCP I is not immunologically related to the PS I reaction center polypeptides (CP I) or LHCP II [6].

On the subject of the possible function of the chlorophyll *a/b* complex of PS I, two hypotheses have been put forward. The first proposed that the complex serves as a light-harvesting antenna of PS I [9], while the second proposed that the complex serves to protect the PS I trap against photodestructive processes [5] in a manner reminiscent of the function of a small antenna complex tightly associated with the reaction center in purple photosynthetic bacteria [10,11].

In the present study, we have investigated the role of the chlorophyll *a/b* complex of PS I in the light-harvesting process by comparing the rate constant for P-700 photooxidation ($K_{P\text{-}700}$) under light-limiting conditions in PS I preparations which contained the chlorophyll *a/b* complex to the $K_{P\text{-}700}$ of PS I preparations devoid of that complex. The effect on $K_{P\text{-}700}$ of recombination of the resolved chlorophyll *a/b* complex with the antenna-depleted PS I complex was also examined. The results indicate that the chlorophyll *a/b* complex associated with PS I can function as an antenna and transfer excitation energy to the reaction center.

A PS I particle was obtained according to the

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Abbreviations: PS I, II, Photosystem I, II; LHCP, light-harvesting chlorophyll-protein complex; CP I, Photosystem I reaction center polypeptides.

procedure described by Mullet et al. [12]. The preparation from our laboratory has been found to contain chlorophyll *a* and *b* in a ratio of approx. 5.5–6 and a chlorophyll/P-700 ratio of approx. 200–280 and has been denoted as PS I-200 [6]. The differences between these properties and those of the Mullet et al. [12] have been discussed in ref. 6. For the resolution of PS I-200 into the chlorophyll *a/b* complex and the P-700-containing complex, the procedure of Haworth et al. [5] was used, except that the sucrose gradients were centrifuged in a Beckman 60 Ti fixed angle rotor at 360 000 × *g* for 3 h instead of in a swinging bucket rotor (100 000 × *g*) overnight as originally described [5]. This procedure resolves three major chlorophyll containing bands. The highest density band contains undissociated PS I-200. The band migrating at the lowest density corresponds to the chlorophyll *a/b* complex of PS I; the middle band corresponds to a PS I fraction containing chlorophyll *a* and P-700, but no chlorophyll *b*. These fractions were stored at −20 °C or used for spectrophotometric measurements as described below. For studies of reconstitution, the PS I preparation depleted of the chlorophyll *a/b* complex and the chlorophyll *a/b* complex were prepared as described by Haworth et al. [5], except that 0.1% Triton X-100 replaced dodecyl β-D-maltoside in the sucrose gradients. The isolated complexes were dialyzed and concentrated (see legend to Fig. 1). Soybean lecithin (100 mg/ml) was sonicated in the dialysis buffer prior to the reconstitution. The reconstitution mixture in a typical assay contained 70 μl of lecithin, 0.45 ml of PS I devoid of the chlorophyll *a/b* complex (0.22 mg Chl/ml), and 0.58 ml of the chlorophyll *a/b* complex (0.34 mg Chl/ml). The mixture was pulse-sonicated at 4 °C for 15 s and used immediately for spectrophotometric measurements. Light-induced absorbance changes associated with the reaction center of PS I (P-700) were measured at 700 nm with a sensitive split-beam spectrophotometer [13] in the laboratory of Prof. A. Melis. Room temperature fluorescence spectra were recorded in a Perkin-Elmer MPF 44B spectrofluorometer in the laboratory of Prof. A. Glazer. Absorbance spectra were recorded at room temperature with a Cary 219 spectrophotometer.

Following mild detergent treatment, the PS I-200 preparation was fractionated on sucrose gradients into two major chlorophyll-containing fractions: the chlorophyll *a/b* complex of PS I and a PS I reaction-center enriched preparation that lacks the chl *a/b* complex. Each of these complexes contains approx. 50% of the total chlorophyll found in the starting PS I-200. The spectral properties of these two complexes are similar to those reported by Haworth et al. [5]: the chlorophyll *a/b* complex had a chlorophyll *a/b* ratio of 3–4 while the reaction-center enriched complex had a ratio grater than 7.5.

The polypeptide profile of PS I-200 and the complexes obtained following fractionation are similar to those previously presented [5–7]. PS I-200 contains polypeptide(s) of about 62 kDa and several polypeptides in the low molecular weight range (smaller than 25 kDa). We have previously shown that the 20 kDa polypeptide in this group is a chlorophyll *a/b*-containing polypeptide [6]. The reaction center polypeptide(s) of approx. 62 kDa are absent from the chlorophyll *a/b* complex. By contrast, the reaction-center enriched PS I preparation recovered from the gradient consists of the 62 kDa polypeptide(s) and three to four low molecular weight polypeptides (smaller than 19 kDa). This fraction lacks all polypeptides present in the chlorophyll *a/b* containing complex (20–25 kDa).

The isolation of two PS I preparations, one retaining the chlorophyll *a/b* complex (PS I-200) and the other lacking that complex, allowed us to test whether the chlorophyll *a/b* complex is involved in a light-harvesting function. Under light-limiting conditions, the rate constant (K) of light absorption by a photosystem is directly proportional to the intensity of the actinic excitation, the absorption cross-section of the light-harvesting pigments and the number of chlorophyll molecules transferring excitation energy to the reaction center [14–16]. If we excite our samples with light that is equally absorbed by chlorophyll *a* and chlorophyll *b*, the measured rate constant, $K_{P\text{-}700}$, for both PS I preparations will be a direct measure of the number of chlorophyll molecules functionally associated with each reaction center. We can thus test whether removal of the chlorophyll *a/b* complex from PS I has any effect on $K_{P\text{-}700}$. If the complex is not involved in energy transfer to the

reaction center of PS I, we would expect a similar rate constant for both the PS I-200 and the reaction-center enriched PS I devoid of the complex. On the other hand, if the chlorophyll *a/b* complex serves as a light-harvesting antenna for the reaction center, we would expect a decrease in the rate constant in the PS I preparation lacking the chlorophyll *a/b* complex that is proportional to the overall number of functional chlorophyll molecules present in the chlorophyll *a/b* complex. The decrease should thus be comparable to the amount of chlorophyll recovered in the form of the complex following fractionation of PS I-200 on sucrose gradients.

Fig. 1A shows the kinetics of P-700 photoconversion for PS I-200 and for the PS I particle depleted of the chlorophyll *a/b* complex. Both samples were excited by low intensity broad band green actinic light which is almost equally absorbed by chlorophyll *a* and *b*. From the amplitude of the absorbance changes, we obtain a chlorophyll/P-700 ratio of about 280 for PS I-200 and of about 140 for the reaction-center enriched PS I preparation depleted of the chlorophyll *a/b* complex. The number of functional chlorophyll molecules associated with each reaction center can be obtained from the rate constant, $K_{P\text{-}700}$, for each PS I preparation. From this measurement, we conclude that the number of functional antenna chlorophyll molecules associated with the reaction center of the PS I particle devoid of the chlorophyll *a/b* complex is about half of that observed in PS I-200.

The determination of $K_{P\text{-}700}$ requires a first-order kinetic analysis of the traces shown in Fig. 1A. A semilogarithmic plot of the kinetics of P-700 photoconversion are presented in Fig. 1B. The slope of the two lines defines the rate constant, $K_{P\text{-}700}$. We calculate a rate constant of 7 s^{-1} for the PS I-200 and a rate constant of 3.5 s^{-1} for the P-700 particle lacking the chlorophyll *a/b* complex. The value observed in the PS I-200 preparation is similar to that observed for P-700 in unfractionated spinach thylakoids under comparable conditions [14–16]. The $K_{P\text{-}700}$ of the PS I particle lacking the chlorophyll *a/b* complex is half of that obtained for PS I-200. Our interpretation is that the number of functional chlorophyll molecules transferring excitation energy to P-700 is reduced

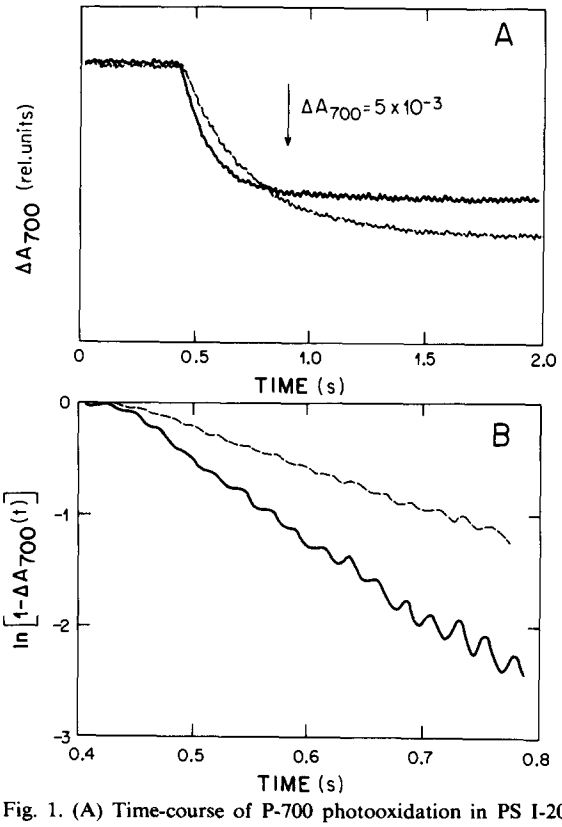

Fig. 1. (A) Time-course of P-700 photooxidation in PS I-200 and the resolved PS I complex. The traces represent an average of four measurements. The reaction mixtures contained 200 μg chlorophyll/ml of PS I-200 and 130 μg chlorophyll/ml of the resolved PS I complex. Samples were dialyzed overnight at 4 °C against 50 mM sorbitol/10mM NaCl/5mM $MgCl_2$/50 mM Tricine (pH 8.0), concentrated against solid sucrose and then diluted to the desired chlorophyll concentration in the above mixture. Spectroscopic measurements were done at 25 °C in the presence of 50 mM ascorbate and 0.2 mM methyl viologen using green actinic light (Corning CS 4-96 and 3-70 filters) at an intensity of 120 $\mu E \cdot m^{-1} \cdot s^{-1}$. (———), PS I-200; (------), antenna-depleted PS I. (B) First-order analysis of the kinetic traces shown in Fig. 1A.

by about half following fractionation of PS I-200, since this treatment effectively removes the chlorophyll *a/b* complex to produce a PS I preparation deficient in an antenna complex. The results are consistent with the chlorophyll *a/b* complex functioning as a light-harvesting chlorophyll antenna for PS I.

In analyzing these results we must take into consideration two possibilities which could have had an effect on the experimental determination of $K_{P\text{-}700}$. The first relates to the fact that limitations

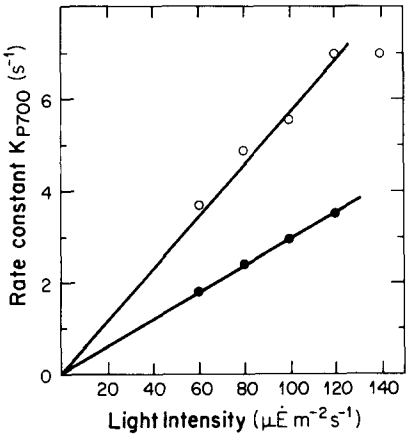

Fig. 2. Light-intensity dependence of P-700 photooxidation in PS I-200 and the resolved PS I complex. Conditions were as in Fig. 1 and the light intensity was varied as indicated. (○——○), PS I-200; (●——●), antenna-depleted PS I.

on the electron acceptor side of PS I (due perhaps to the use of detergents in the preparation of the various fractions studied) could in fact have been responsible for a smaller $K_{P\text{-}700}$ associated with the PS I particle depleted of the chlorophyll *a/b* complex. As shown in Fig. 2, we have found that the rate constants ($K_{P\text{-}700}$) for both PS I preparations are linear over the range of light intensities used. We interpret these results to indicate that the limiting factor in these experiments is the amount of light absorbed by the functional light-harvesting pigments and not electron transfer into or out of the reaction center complex. The second possibility relates to a possible dislocation of pigments in the complexes as a result of detergent treatment producing a non-functional state, and this possibility can be examined through an analysis of the fluorescence properties of the various complexes. The isolated chlorophyll *a/b* complex has a room temperature fluorescence maximum at 676 nm (see Ref. 5) and a relative fluorescence yield several-fold higher than that of PS I-200 or the PS I particle lacking this complex. Moreover, the relative fluorescence yield of the PS I particle lacking the chlorophyll *a/b* complex is less than that of the PS I-200 (data not shown). These results indicate that, in the case of the PS I particle lacking the chlorophyll *a/b* complex, the functional association of pigment molecules in the complex has not been altered as compared with the starting material (PS I-200) since a marked dislocation of chlorophylls would have resulted in larger relative fluorescence yields. The higher yield observed in the isolated chlorophyll *a/b* complex originates because a trap, such as P-700, which can efficiently serve to quench fluorescence, is absent.

In addition to the above analysis of antenna sizes, we have succeeded in the partial reconstitution of the chlorophyll *a/b* complex-depleted PS I preparation with the isolated chlorophyll *a/b* complex. Fig. 3A shows the kinetic traces of the photooxidation of P-700 while Fig. 3B presents a semi-logarithmic analysis. It is observed that after reconstitution $K_{P\text{-}700}$ increased significantly. About half of the original antenna was probably reconstituted as manifested by the recovery of 50% of the rate constant in the PS I devoid of the chlorophyll *a/b* complex. The reconstituted PS I preparation also shows strict light intensity dependency on its value of $K_{P\text{-}700}$ (data not shown). Thus, the increase in $K_{P\text{-}700}$ as a result of reconstitution most likely reflects an increase in the functional antenna size of PS I reaction center.

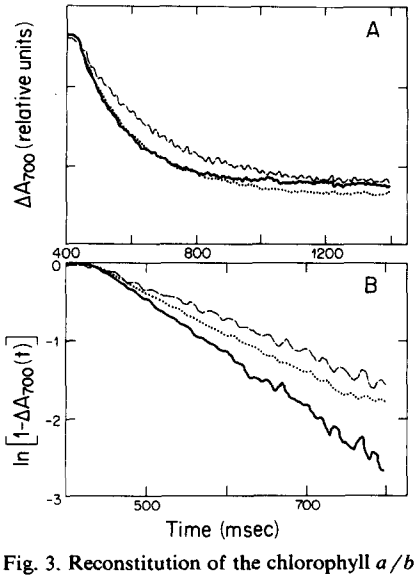

Fig. 3. Reconstitution of the chlorophyll *a/b* complex with the resolved PS I complex. The time-course of P-700 photooxidation in the reconstituted PS I complex was analyzed as described in Fig. 1 using the same reaction mixture. Also shown in the traces are the time-courses for the antenna-depleted PS I complex and PS I-200. (———), PS I-200; (— —), PS I (antenna depleted); (------) PS I (reconstituted). (A) Kinetics of P-700 photooxidation; (B) first order analysis of the kinetic traces in (A).

The present work demonstrates a decrease in the rate of P-700 photooxidation in the PSI particle devoid of the chlorophyll *a/b* complex compared with the starting material (PS I-200). Our interpretation is that the loss of the chlorophyll *a/b* complex during fractionation of PS I-200 results in a PS I preparation with a decreased functional light-harvesting antenna. The $K_{P\text{-}700}$ of the PS I preparation devoid of the chlorophyll *a/b* complex is 50% smaller than that of the PS I-200, and the original rate of $K_{P\text{-}700}$ can be substantially restored by mixing the isolated chlorophyll *a/b* complex with the antenna-depleted PS I preparation under suitable conditions. The decrease in $K_{P\text{-}700}$ reflects the distribution of chlorophyll between the chlorophyll *a/b* complex and the PS I preparation devoid of the chlorophyll *a/b* complex following fractionation of PS I-200, since approx. 50% of the chlorophyll is recovered in the chlorophyll *a/b* complex while the remaining chlorophyll is recovered in the antenna-depleted PS I preparation. We believe that the chlorophyll *a/b* complex is not a minor chlorophyl-containing component, but is a PS I antenna complex that represents about 40–50% of the total chlorophyll associated with PS I in vivo.

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