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THE RATIO OF THE TWO LIGHT REACTIONS AND THEIR COUPLING IN CHLOROPLASTS

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SUMMARY

The kinetics of the absorbance changes of chlorophyll a_I (*P*-700) and plastoquinone induced by xenon flashes of saturating intensity were studied in spinach chloroplasts.

1. The total amount of chlorophyll a_I is compared with that amount being reduced via the rate-limiting step between the light reactions. This is based on the amplitudes of the absorbance changes of chlorophyll a_I after chemical reduction and after a group of flashes following far-red preillumination. It is concluded that only 75 % of chlorophyll a_I is coupled to chlorophyll a_{II} via linear electron transport and that the remaining 25 % is functionally isolated.

2. A ratio of 0.85 for coupled chlorophyll a_I to chlorophyll a_{II} is estimated from the time course of the absorbance changes of plastoquinone and chlorophyll a_I in two independent ways.

3. The oxygen yield per flash is used to calculate the difference extinction coefficient of chlorophyll a_I at the maximum of the red absorbance band in spinach chloroplasts: $\Delta\varepsilon_{703} = (6.7 \pm 0.7) \cdot 10^4\ M^{-1} \cdot cm^{-1}$. The assumption of a quantitative electron transfer from water via plastoquinone to coupled chlorophyll a_I is supported by the same extinction coefficient reported by Hiyama and Ke for Photosystem I particles.

The location and function of the different chlorophylls a_I is discussed in detail.

INTRODUCTION

The series arrangement of two photochemical light reactions in the photosynthetic electron transport chain [1] has been established by spectroscopic measurements of different absorbance changes associated with electron carriers [2–4]. A pool of plastoquinone functions as a link [5, 6] between the reaction centers of

Abbreviations: Chl-a_I, chlorophyll a_I; Chl-a_{II}, chlorophyll a_{II}; PQ, plastoquinone; PQH_2, plastohydroquinone.

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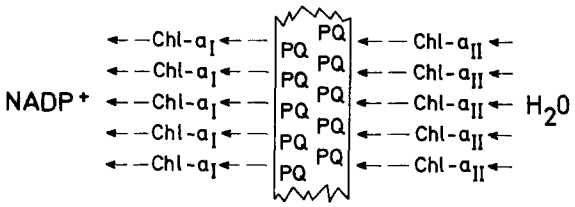

Fig. 1. Simplified scheme of linear electron transport showing the coupling of different light reactions via a common pool of plastoquinone [6, 11].

light Reaction I, chlorophyll a_I (*P*-700) [7, 8], and the reaction centers of light Reaction II, chlorophyll a_{II} (*P*-680) [9, 10]. A coupling of at least ten electron transport chains *via* a common pool of plastoquinone has been shown by the absorbance changes of plastoquinone [6] and chlorophyll a_I [11]. The common pool of plastoquinone collects electrons from light Reaction II and distributes them to light reaction I as illustrated in Fig. 1. Therefore the chlorophyll a_I to chlorophyll a_{II} ratio in linear electron transport can differ from one, the value of isolated electron transport chains.

Previous attempts to determine the ratio of the two light reactions were based on a comparison of the amounts of chlorophyll a_I and chlorophyll a_{II} found in relation to total chlorophyll. The amount of chlorophyll a_{II} can only be calculated from the oxygen yield per saturating short flash and is about one chlorophyll a_{II} per 500 chlorophylls [12]. Chlorophyll a_I, on the other hand, is directly detectable by its absorbance change. However, the estimation of the amount of chlorophyll a_I suffered from the uncertainty in the numeric value of its difference extinction coefficient [13–18].

This also affects the previously estimated stoichiometry of 1:1 between chlorophyll a_I oxidation and NADP reduction [13]. In addition, some doubt about the involvement of chlorophyll a_I in linear electron transport from water to NADP has been raised [19].

Another approach to the evaluation of the ratio of the light reactions was based on the finding of an approximately equal contribution of the two light reactions to the electrochromic absorbance change at 515 nm in spinach chloroplasts [15]. In *Chlorella* a higher contribution of light Reaction I than of light Reaction II to this absorbance change was found [20].

The existence of functionally different light reactions which could yield different ratios depending on the experimental conditions is a further important aspect. Treatment of spinach chloroplasts with detergents [21, 22] or by mechanical forces [22, 23] separates not only Photosystem I and Photosystem II but also breaks the stroma lamellae and the end membranes from the grana stacks. Different authors [23–25] have shown that stroma lamellae contain only Photosystem I while grana lamellae contain the two photosystems. But the function of these structurally different Photosystems I remains still unknown.

This paper deals with two methods for determining the ratio of coupled chlorophyll a_I to chlorophyll a_{II} from the kinetics of the absorbance changes of plastoquinone and chlorophyll a_I. From this ratio, and from the oxygen yield per flash the difference extinction coefficient of chlorophyll a_I in intact chloroplasts can be calculated. Furthermore, from the amounts of chlorophyll a_I being reduced by

electrons either from water or from ascorbate mediated by phenazine methosulfate, it is shown that only 75 % of the total chlorophyll a_I is coupled to chlorophyll a_{II}.

MATERIALS AND METHODS

Class II chloroplasts (type C) and hypotonically broken chloroplasts (type E) were prepared from spinach as described previously [18] and either used fresh or stored under liquid N_2 in the presence of 5 % dimethylsulfoxide until use. Class I chloroplasts (type B) were isolated from greenhouse grown spinach similar to the method of Hall et al. [26]. Grana were prepared with a French pressure cell according to the method described by Sane et al. [24]. Chlorophyll was determined using the spectrophotometric method of Arnon [27]. KCN treated chloroplasts were prepared as described by Izawa et al. [28]. Reaction mixtures contained: chloroplasts at a concentration of 10 μM chlorophyll; 20 mM *N*-tris-(hydroxymethyl)methylglycine/NaOH buffer at pH 7.2; 20 mM KCl; 1 mM $MgCl_2$; 10 μM benzylviologen as electron acceptor; 1 μM gramicidin D for uncoupling. Reaction mixtures with KCN treated chloroplasts additionally contained 3.3 μM phenazine methosulfate and 6.7 μM sodium ascorbate. The temperature was 20–22 °C.

The absorbance changes of plastoquinone at 265 nm and chlorophyll a_I at 705 nm were measured simultaneously with a repetitive flash photometer with double beams similar to that described in ref. 9. The chloroplast suspension was illuminated for 4.5 s with far-red light (720 nm, $\Delta\lambda = 15$ nm) at an intensity of $2 \cdot 10^4$ ergs $\cdot$ $cm^{-2} \cdot s^{-1}$ followed by a dark period of 0.5 s during which the absorbance changes were induced by xenon flashes of blue light of saturating intensity (Schott filters: BG 23, 6 mm, and KG 2, 2 mm). Consecutive 5-s periods with this illumination regime were repeated to average the signals. The duration of the flashes was 25 μs at half of the peak and 65 μs including the short tail. In the special measurements with KCN treated chloroplasts, far-red illumination was omitted and the frequency of the repetitive flashes was 1 Hz. Between 64 and 256 signals were averaged in a Fabri-Tek 1072. The sample was changed after every 64 signals. The electrical bandwidth was 5 kHz. The optical path length of the cuvette was 20 mm, the intensity of the monitoring light less than 50 ergs $\cdot$ $cm^{-2} \cdot s^{-1}$, and the optical bandwidth $\Delta\lambda = 3$ nm at 705 nm and 2.5 nm at 265 nm.

RESULTS

The ratio of the light reactions coupled in linear electron transport

The simultaneous time courses of the absorbance changes of plastoquinone at 265 nm and of chlorophyll a_I at 705 nm induced by two flashes 0.1 s after preillumination with far-red light are shown in Fig. 2. Plastoquinone is rapidly reduced and reoxidized with a half-life time of 24 ms (average value 20 ms), which is the half-life time of the rate-limiting step of linear electron transport [29]. The second flash is spaced at an interval of 205 ms to allow complete relaxation of the absorbance changes. The time course of plastoquinone oxidation induced by the second flash is the same as that induced by the first flash. The preillumination with far-red light of nonsaturating intensity oxidizes all electron carriers between the two light reactions except about 30 % of chlorophyll a_I [30]. The first flash oxidizes this portion of

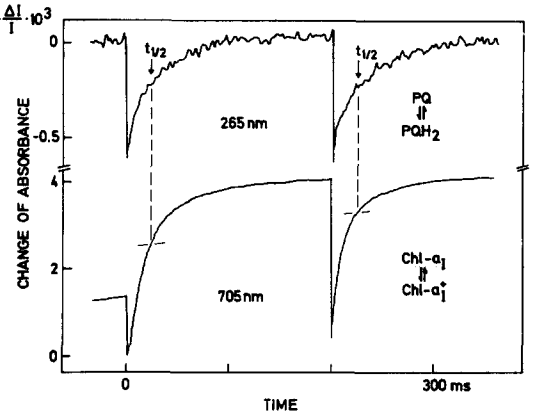

Fig. 2. Absorbance changes of plastoquinone at 265 nm and of chlorophyll a_I at 705 nm as a function of time induced by two flashes spaced at 205 ms. The indicated half-life times were found by a semi-log plot of the time courses at 265 nm. Both traces are the average of 265 simultaneously measured signals.

chlorophyll a_I. The following time course represents the reduction of chlorophyll a_I by electrons from water via the rate-limiting step [31]. In the second flash the reduced chlorophyll a_I is again oxidized.

The far-red preillumination not only oxidizes the electron carriers between the light reactions but also keeps the oxygen evolving system in its steady state [32]. Therefore and since the flash duration is much shorter than the 0.6 ms half-life time on the oxidizing side of light Reaction II [33], the number of electrons produced by a saturating flash should equal the number of chlorophyll a_{II}. Taking this into account, it is possible to calculate the ratio of chlorophyll a_I to chlorophyll a_{II} from the reaction kinetics shown in Fig. 2 without knowing the extinction coefficients. The following assumptions have to be made:

(1) All electrons from water are transferred via plastoquinone to chlorophyll a_I.

(2) The oxidation reduction equilibrium between chlorophyll a_I and its immediate donors (plastocyanin and cytochrome f) is well towards chlorophyll a_I. This is supported by the known midpoint potentials of these electron carriers [34–37].

As a consequence of the first assumption the amplitude of the fast reduction of plastoquinone after the flash should be proportional to the total amount of chlorophyll a_{II}. The amplitude of the reoxidation kinetics of plastoquinone at any given time indicate the number of electrons released via the rate-limiting step to the oxidized electron carriers on the side of light Reaction I. For example after the first half-life time (24 ms in Fig. 2) 50 % of the electrons are released. According to the second assumption the donors should stay oxidized until most of chlorophyll a_I is reduced. From the kinetics shown in Fig. 2 the amount of chlorophyll a_I reduced 24 ms after the first flash is found to be 63 %. Obviously one half of the electrons produced by chlorophyll a_{II} are capable of reducing more than one half of chlorophyll a_I. This is only possible if the total number of chlorophyll a_I coupled to chlorophyll a_{II} is smaller than the total number of chlorophyll a_{II}. Dividing the corresponding amounts (50 % : 63 %) gives the ratio of chlorophyll a_I : chlorophyll $a_{II} = 0.80$.

The percentage of reduced chlorophyll a_I (63 %) was related to the maximal amount of chlorophyll a_I that can be reduced via plastoquinone. The corresponding maximal amplitude (see Fig. 4 left) can only be determined after a group of saturating flashes [18] or after illumination with strong light [38]. As shown by the method of flash titration, four flashes spaced at 1.6 ms accumulate about 2.4 electrons per light Reaction II in the pool of plastoquinone which are sufficient to reduce chlorophyll a_I completely [18]. A complete reduction of chlorophyll a_I within the experimental error is also expected if an equilibration of these electrons between chlorophyll a_I and its donors according to the midpoint potentials is assumed.

The amount of electrons produced by chlorophyll a_{II} in one flash exceeds the electron capacity of chlorophyll a_I. Therefore the immediate donors of chlorophyll a_I must be partially reduced if all electrons have passed through the rate-limiting step. The electrons stored in these donors after the first flash are expected to rapidly reduce the oxidized chlorophyll a_I in 20 μs and 200 μs [39, 40] after a second flash. This leads to the second method of evaluating the ratio of chlorophyll a_I to chlorophyll a_{II} from the absorbance changes of chlorophyll a_I only.

The identical time course for the reoxidation of plastoquinone after the first and second flash (Fig. 2) indicates an identical electron transport from water to Photosystem I via the rate-limiting step. Nevertheless, after the first half-life time of the reoxidation of plastoquinone the amount of reduced chlorophyll a_I is obviously greater after the second flash than after the first one. A part of chlorophyll a_I should be rapidly reduced by electrons from plastocyanin and cytochrome *f*, in addition to the electrons released from plastoquinone as postulated. Due to the insufficient time-resolution used in this experiment it is not possible to observe these fast kinetics. However, their amplitude can be derived from the time courses of chlorophyll a_I on the assumption that electrons from plastoquinone preferentially reduce oxidized chlorophyll a_I even if a part of chlorophyll a_I is previously reduced. This is indeed found and is demonstrated in Fig. 3. The second half of the chlorophyll a_I trace in Fig. 2 was shifted downwards in the averager for the best fit of the initial portion of both time courses. The signals were recorded directly one over the other on an expand-

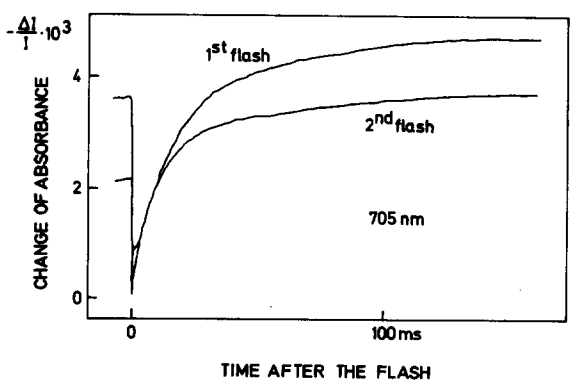

Fig. 3. Absorbance changes of chlorophyll a_I at 705 nm from Fig. 2 on a twice expanded time scale. The recorded time course after the second flash was shifted downwards and then overlapped with that recorded after the first flash. For details see text.

ed time scale. The overlapping of one third of the amplitudes in the initial portion satisfies the expectation that chlorophyll a_I is reduced via the rate-limiting step without the influence of the preceding fast electron transfer after the second flash. But the reduction of chlorophyll a_I is completed earlier after the second flash than after the first one as can be seen from the diverging time courses. The vertical difference after relaxation of the kinetics must agree in amplitude to the fast kinetics after the second flash. It equals 25 % of the total amplitude of chlorophyll a_I. This should correspond to the amount of plastocyanin and cytochrome *f* being reduced after the first flash. After the first flash 92 % of chlorophyll a_I is reduced at the same time (cf. also ref. 18). In total 117 % of chlorophyll a_I could have been reduced by the electrons produced by chlorophyll a_{II} in the first flash. The ratio of chlorophyll a_I : chlorophyll $a_{II} = 0.85$ results from the reciprocal (100 : 117) and is in good agreement with the previous value. The reduced amounts of chlorophyll a_I and its donors confirm that the equilibrium of the electron transfer is well towards chlorophyll a_I.

The ratio of chlorophyll a_I to chlorophyll a_{II} of eight different preparations of chloroplasts was calculated from the reduced amount of chlorophyll a_I after the first half-life of the reoxidation of plastoquinone as well as from the amount of chlorophyll a_I and its immediate donors reduced after the first flash. The average and its maximal errors is

chlorophyll a_I : chlorophyll $a_{II} = 0.85 \pm 0.05$.

No significant differences were found for chloroplasts (Class II) or broken chloroplasts used fresh or stored under liquid N_2 before use.

The difference extinction coefficient of chlorophyll a_I in chloroplasts

The difference extinction coefficient of a substance, $\Delta\varepsilon$, can be calculated from the absorbance change, if the change of its concentration is known. The concentration of chlorophyll a_I coupled to chlorophyll a_{II}, $c_{\text{Chl-}a_I}$, can be computed by the product of the ratio of chlorophyll a_I to chlorophyll a_{II}, Chl-a_I : Chl-a_{II}, the ratio of chlorophyll a_{II} to total chlorophyll, Chl-a_{II} : Chl, and the concentration of total chlorophyll c_{Chl}

$$c_{\text{Chl-}a_I} = \frac{\text{Chl-}a_I}{\text{Chl-}a_{II}} \cdot \frac{\text{Chl-}a_{II}}{\text{Chl}} \cdot c_{\text{Chl}}$$

The ratio of chlorophyll a_{II} to total chlorophyll was estimated from the maximal oxygen yield per flash and yielded ratios of about 1 : 500 in accordance with previous results [12, 29]. The maximal amplitude of the absorbance change at 705 nm after a group of flashes (see above and Fig. 4 left) should correspond to the maximal change in the concentration of oxidized chlorophyll a_I due to electrons from water. It was used to estimate the difference extinction coefficient of chlorophyll a_I at 705 nm. The average of five preparations of spinach chloroplasts is $\Delta\varepsilon_{705} = 6.4 \cdot 10^4\ \text{M}^{-1} \cdot \text{cm}^{-1}$. The difference spectra of the absorbance changes induced by one flash were measured with and without far-red preillumination, respectively. They peaked at 703 nm in accordance with previous results [31, 41, 42]. However, depending on the species the location of the maximum occurs at slightly different wavelength (see e.g. [17, 43–45]). With respect to the slightly larger amplitude at 703 nm compared to that at 705 nm

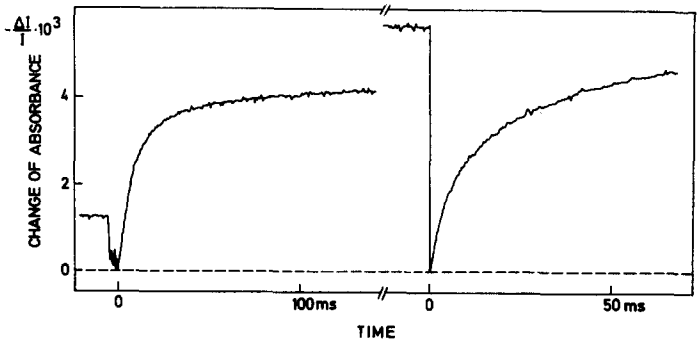

Fig. 4. Absorbance changes of chlorophyll a_I at 705 nm as a function of time. Left, time course induced by a group of four flashes 0.1 s after preillumination for 4.5 s with far-red light. The time between the flashes was 1.6 ms. Right, time course induced by one flash in the presence of reduced phenazine methosulfate after previous incubation of the chloroplasts with KCN. Both traces are the average of 64 signals.

the difference extinction coefficient of chlorophyll a_I at the maximum of the red absorbance band in spinach chloroplasts with its maximal error is

$$\Delta\varepsilon_{703} = (6.7 \pm 0.7) \cdot 10^4\ M^{-1} \cdot cm^{-1}$$

Chlorophyll a_I not coupled to chlorophyll a_{II}

The experiments reported above cover the chlorophyll a_I molecules connected to chlorophyll a_{II} as a consequence of the alternating excitation with System I light (far-red) and flashes of System I and System II light (blue) in the presence of benzylviologen. The maximal amplitude of the absorbance change at 705 nm under these conditions is shown in Fig. 4 left. It should be emphasized that groups of more than four flashes during which larger amounts of electrons are accumulated in the pool of plastoquinone do not increase the amplitude [18]. Nevertheless, even in intact chloroplasts some chlorophyll a_I may not be coupled to linear electron transport. If this is the case, the absorbance changes of this chlorophyll a_I can only be detected if it is reduced again during the dark period between the repetitive flashes. This can be realized by the addition of sodium ascorbate plus phenazine methosulfate. Reduced phenazine methosulfate appears to be capable of donating electrons not only to the electron carriers between plastoquinone and chlorophyll a_I but also directly to chlorophyll a_I [28]. Therefore the fast electron transfer from these electron carriers to oxidized chlorophyll a_I after a flash can be blocked with KCN [28]. This will be presented in a subsequent paper. Blocking of the fast reduction of chlorophyll a_I increases the accuracy of the resolution of the total amplitude because of a lower time resolution necessary. Fig. 4 right shows the absorbance change of chlorophyll a_I engaged in phenazine methosulfate mediated electron transport. The oxidation in the flash is followed by a slow reduction of chlorophyll a_I. The half-life time was found to be a function of the concentration of phenazine methosulfate in agreement with previous results [31]. However, the amplitude is the same when phenazine methosulfate is replaced by 50 μM 2,6-dichlorphenolindophenol and should be due to the total amount of chlorophyll a_I in the chloroplasts. Related to this total amount, the

amount of chlorophyll a_I coupled to chlorophyll a_{II} is calculated from the amplitude of the absorbance changes in Fig. 4. The average value of five different preparations is 75 % (±5 %). No significant difference is found for whole (Class I and Class II) or hypotonically broken chloroplasts. The resulting ratio of total chlorophyll a_I to chlorophyll a_{II} is 1.13.

DISCUSSION

The above experiments have led to the identification of functionally different chlorophylls a_I. To compare the results with other findings one has to refer to this aspect. The higher contribution of light Reaction I to the electrochromic absorbance change found by Joliot and Delosme [20] compared to the result of Schliephake et al. [15] may be caused not only by the different species but also by the excitation conditions. In spinach chloroplasts the endogenous dark reduction of oxidized chlorophyll a_I is completed within 5 min [46]. From this we suggest that in contrast to the experiment with isolated chloroplasts using a high repetition rate of the flashes [15] in the dark adapted *Chlorella* [20] the total chlorophyll a_I contributed to the electric field across the membrane. Thus the ratios of 1.5 and 4 estimated by Joliot and Delosme seem to support our finding of a ratio of total chlorophyll a_I to chlorophyll a_{II} greater than one.

To estimate the ratio of coupled chlorophyll a_I to chlorophyll a_{II} the number of chlorophyll a_{II} was assumed to be equal to the number of electrons produced after a saturating flash. But 10 % of the photoevents of chlorophyll a_{II} may be misses as concluded from the damping of the flash-yield oscillation of oxygen [47]. Although the time course of the flash intensity (see Materials and Methods) differs from that of Kok et al. [48] and of Joliot et al. [49] we suggest that the percentage of double hits approximately compensates that of the misses. A different number of double hits would cause a small difference between the estimated and the true ratio of coupled chlorophyll a_I to chlorophyll a_{II}. However, the value of the difference extinction coefficient of chlorophyll a_I is not affected by possible misses and double hits because these effects are eliminated by the product of the estimated ratio of chlorophyll a_I to chlorophyll a_{II} and the oxygen yield per flash.

The difference extinction coefficient of chlorophyll a_I in whole spinach chloroplasts, $\Delta\varepsilon_{703} = 6.7 \cdot 10^4\ M^{-1} \cdot cm^{-1}$, is estimated with respect to those chlorophyll a_I being rapidly reduced by electrons from water (Fig. 4 left). The calculation is based on the assumption of a quantitative electron transfer from water via plastoquinone to chlorophyll a_I. A loss of electrons to an unknown electron path would be indicated by a greater value of the difference extinction coefficient determined by other methods. However, in subchloroplast particles from spinach and *Anabaena* enriched in chlorophyll a_I almost the same values ($\Delta\varepsilon_{700} = 6.4 \cdot 10^4\ M^{-1} \cdot cm^{-1}$ and $\Delta\varepsilon_{701} = 7.0 \cdot 10^4\ M^{-1} \cdot cm^{-1}$, respectively) were calculated from the absorbance change of *N,N,N',N'*-tetramethylphenylenediamine which performs a simple cyclic electron flow around light Reaction I in these particles [17]. The agreement of the values within the experimental error can be taken as evidence for the transfer of all electrons produced by chlorophyll a_{II} via plastoquinone to chlorophyll a_I. This excludes all models of the linear electron transport chain assuming another light

reaction functioning in parallel [46] or in place of chlorophyll a_I [19, 50] in linear electron transport.

Recently we have estimated the lower limit of the difference extinction coefficient of chlorophyll a_I by comparing the oxygen yield per flash with the amounts of cytochrome *f* and chlorophyll a_I reduced after one flash ($\Delta\varepsilon_{705} \geqslant 5.4 \cdot 10^4\ M^{-1} \cdot cm^{-1}$) [18]. In this investigation it was not possible to take into account the reduction of another donor of chlorophyll a_I other than cytochrome *f* which would otherwise have increased the calculated value. This must be postulated if the 0.21 electron equivalents per chlorophyll a_{II} reducing the donors of chlorophyll a_I are compared with the 0.07 electron equivalents reducing cytochrome *f* after one flash [18].

Location and function of different chlorophylls a_I

Functionally different chlorophyll a_I can be discriminated if the velocity of the reduction by electrons from water is much faster than by cyclic electron transport. In spinach chloroplasts the quantitative electron transfer from water occurs to only 75 % of the total chlorophyll a_I. We conclude that the remaining 25 % of total chlorophyll a_I is not coupled to chlorophyll a_{II}. A possible location in the photosynthetic membrane can be suggested from the amounts of Photosystem I and Photosystem II separated from chloroplasts. Arntzen et al. [25] fractionated stroma lamellae containing only Photosystem I and grana membranes consisting of 51 % of Photosystem II and of 35 % of Photosystem I on the basis of total chlorophyll. Quantum-yield measurements of the two photosystems showed that the chlorophyll of whole chloroplasts is about equally distributed between the two photosystems [51]. If this holds for the grana membranes and if detergents separate the bulk chlorophylls of the photosystems the ratio of Photosystem I to Photosystem II is 0.7 in the grana membranes. The ratio of 0.85 of the coupled light reactions may be an indication for a favoured coupling of the chlorophyll a_I in the grana. Sane et al. [24] and Arntzen et al. [25] have measured the percentages of total chlorophyll as well as the ratios of chlorophyll to chlorophyll a_I of their fractions from spinach chloroplasts. From their data it is possible to estimate the amount of chlorophyll a_I which is believed to originate from the stroma lamellae and the end membranes: 30 % and 35 % of the total amount of chlorophyll a_I is obtained, respectively. The rather good agreement with the value of 25 % (± 5 %) of chlorophyll a_I found to be isolated from light Reaction II suggests their location in the stroma lamellae. The small difference between the values would easily be explained if chlorophyll a_I in the end membranes of the grana stacks is coupled to light Reaction II but removed by French press treatment. An interaction of Photosystem I in the stroma lamellae with Photosystem II via a diffusable electron carrier [24] seems to be improbable.

The electron micrographs of Hall et al. [52] showing sites of ferricyanide reduction in the grana as well as in the stroma lamellae are not consistent with the suggested location of the functionally different chlorophylls a_I. Therefore grana membranes obtained by French press treatment according to the method of Sane et al. [24] were examined. But even if the chloroplasts were passed through the French press only once at a low pressure (4000 lb/inch^2), the absorbance changes at 705 nm allowed no conclusive interpretation. The treatment causes a damage of the coupling of chlorophyll a_I which may be due to the partial loss of plastocyanin [23, 53, 54].

The coupling of a larger number of chlorophyll a_{II} via a common pool of

plastoquinone with a smaller number of chlorophyll a_I may be explained by the reaction times of the light reactions. Light reaction II produces electrons with an average half-life time of 0.6 ms [33] while the half-life time of the electron transfer to chlorophyll a_I is at least three times faster (200 μs and 20 μs). Furthermore in strong light the oxygen production is limited by the rate-limiting oxidation of plastoquinone [29]. Consequently the coupled chlorophyll a_I molecules are able to turn-over much faster than light Reaction II. They can drive not only the linear but also the cyclic electron transport. The 25 % of total chlorophyll a_I which may be located in the stroma lamellae may function exclusively in a cyclic electron transport, e.g. with ferredoxin [55] to produce ATP.

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