Biochimica et Biophysica Acta, 635 (1981) 111—120

BBA 48011

QUANTUM EFFICIENCY AND ANTENNA SIZE OF PHOTOSYSTEMS II$_\alpha$, II$_\beta$ AND I IN TOBACCO CHLOROPLASTS

A.P.G.M. THIELEN and H.J. VAN GORKOM

Department of Biophysics, Huygens Laboratory, University of Leiden, P.O. Box 9504, 2300 RA Leiden (The Netherlands)

(Received August 4th, 1980)

Key words: Photosystem II; Photosystem I; Quantum yield; Antenna size; (Tobacco chloroplast)

Summary

Reaction center concentrations were determined in chloroplasts of tobacco, cv John William's Broadleaf, and its mutants Su/su and Su/su var. Aurea. Quantum yields of the primary reactions of Photosystems I, II$_\alpha$ and II$_\beta$ (Melis, A. and Homann, P.H. (1975) Photochem. Photobiol. 21, 431—437) were obtained by measurement of their rate constants and the absorbed energy, under conditions where all three photosystems operated simultaneously and produced almost irreversibly a single charge separation.

The concentrations and reaction rates of the photosystems were different in chloroplasts from the wild type and the mutants, but in chloroplasts of each type of plant used essentially all quanta absorbed by chlorophyll caused a charge separation in PS I, PS II$_\alpha$ or PS II$_\beta$. Since the quantum efficiency of each photosystem was close to one, kinetic differences between the photosystems and between different kinds of chloroplasts were only due to differences in antenna size. From the rate constants the number of chlorophyll molecules in the antenna of each photosystem could be calculated. It is argued that PS II$_\alpha$ and PS II$_\beta$ must be different, independent structures.

Introduction

In recent years evidence has accumulated for the existence of a second type of Photosystem II, PS II$_\beta$, which differs from the normal PS II$_\alpha$ by its appar-

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Abbreviations: PS, Photosystem; P-700, primary donor of Photosystem I; Q, primary acceptor of Photosystem II; Chl, chlorophyll; DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea.

ently slower reaction rate in continuous light [1—3]. Differences in antenna properties [3,4] as well as electron transport properties [1,3] between PS II_α and PS II_β have been reported. It still is not clear, however, whether the slower reaction rate of PS II_β is due to a smaller pigment antenna or to a low quantum yield. Since the latter possibility would cast some doubt on its physiological significance, it seemed important to determine the quantum yield of PS II_β.

We used chloroplasts of tobacco, cv John William's Broadleaf, and its mutants Su/su and Su/su var. Aurea [5], which differ strongly in the relative amounts of PS II_α and PS II_β [6]. We determined the partial quantum yields of the primary reactions of all three photosystems from their concentrations, the rate constants of their primary reactions and the absorbed light intensity. A new method to correct for the effect of flattening on absorbance changes was applied, which was insensitive to the differences in pigment composition. In chloroplasts from each type of plant, the total quantum yield of the three photosystems together turned out to be close to unity. Therefore all three photosystems must function with high efficiency and absolute antenna sizes could be given for each photosystem in each kind of material.

Methods

a. Apparatus and material

The plants 'wild type' *Nicotiana tabacum*, cv John William's Broadleaf, and the green-yellow mutant Su/su were grown in the laboratory at 20—25°C and a light intensity of approximately 4000—5000 lux. The yellow mutant Su/su var. Aurea was grown in a greenhouse. Chloroplasts were isolated as described in Ref. 2. Chlorophyll concentration and the Chl *a*/Chl *b* ratio were determined according to Arnon [7]. Light-induced fluorescence and absorbance changes were measured as described earlier [6]. The reaction mixture always contained chloroplasts suspended in the isolation medium (0.4 M sucrose, 50 mM *N*-tris(hydroxymethyl)methylglycine, 10 mM KCl and 5 mM $MgCl_2$, pH 7.8) at a chlorophyll concentration of 50 μM. The optical pathlength of the sample was 1.2 mm. From the absorbance and scattering spectra, measured for each batch of chloroplasts on a Cary 14 or a Shimadzu MPS 5000 spectrometer, spectra corrected for scattering losses were calculated as in Ref. 8.

b. Flattening correction

Quantitative calculation of absorbance and absorbance changes measured in a suspension of strongly absorbing particles requires a correction for the so-called flattening effect [9—11]. The amplitude of absorbance changes is reduced by a differential flattening factor f'. In first approximation $1 - f'$ is proportional to the absorbance of the suspension [10]. In spinach chloroplasts, where the smallest differential flattening factor is about 0.3, this proportionality should hold within about 2%. We therefore assume that the following relation holds

$$1 - f'(\lambda) = k \cdot A_{susp}(\lambda) \tag{1}$$

The proportionally constant k may then be determined from the ratio ($Q_{f'}$) of the differential flattening factors at two wavelengths λ_1 and λ_2 according to the relation

$$k = \frac{Q_{f'} - 1}{A_{\mathrm{susp}}(\lambda_2) \cdot Q_{f'} - A_{\mathrm{susp}}(\lambda_1)} \tag{2}$$

$Q_{f'}$ is found by measurement of absorbance changes of a membrane component with a known flattening-free difference spectrum:

$$Q_{f'} = \frac{A(\lambda_1)_{\mathrm{susp}}}{A(\lambda_2)_{\mathrm{susp}}} \cdot \frac{\Delta\epsilon(\lambda_2)}{\Delta\epsilon(\lambda_1)}$$

Suitable components in chloroplasts are P-700 and cytochrome *f*. Their difference spectra are given in Refs. 12 and 13. Both spectra have peaks in the blue region, where flattening is severe, and in a region where little flattening occurs, around 554 nm for cytochrome *f* and 700 nm for P-700. We measured the absorption changes at 700 nm, at 400—445 nm and at 530—575 nm, induced by continuous illumination in the sample in the presence of 10 μM DCMU, 2 mM hydroxylamine, 30 μM of gramicidin D and 50 μM of methylviologen. The remaining light-induced absorption changes reflect the functioning of the oxidizing side of PS I with oxidation of P-700, cytochrome *f* and plastocyanin and reduction of cytochrome *b*-563 [14]. In the presence of 1 mM ascorbate the changes were reversible in the dark.

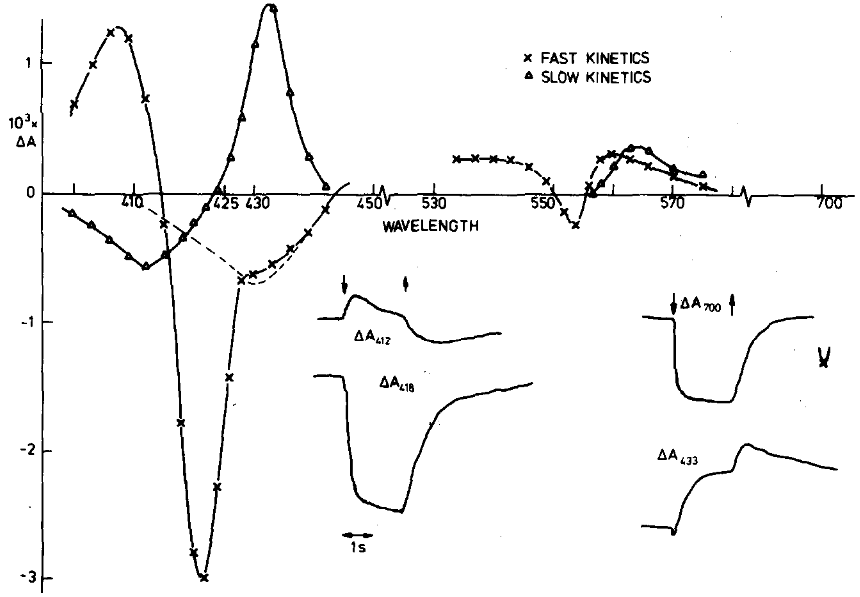

Fig. 1. Parts of the difference spectrum of Su/su var. Aurea chloroplasts pertinent to the measurement of the differential flattening factors. The slow light-on and light-off changes (see inset) are caused by the reduction of cytochrome. *b*-563. The fast changes represent pure P-700 oxidation in the red region, in the blue region the oxidation of P-700 and cytochrome *f*, and oxidation of P-700, cytochrome *f* and plastocyanin in the green region. The dashed line is an estimate of the contribution of P-700 to the blue part of the difference spectrum. With the aid of Fig. 2B f'_{325} was calculated to be 0.93 from the results shown here.

The changes of cytochrome *b*-563 could normally be distinguished kinetically from those caused by the other components (see Fig. 1). To be sure we determined cytochrome *f* changes at 423.5 nm, an isosbestic wavelength of cytochrome *b*-563. In the green region the formula $\Delta A_{556} - \frac{1}{2}(\Delta A_{543} + \Delta A_{560})$ effectively cancelled changes of cytochrome *b*-563 [15] and left only a very small contribution of P-700 and plastocyanin.

In a first approach we separated the changes of P-700 from those of cytochrome *f* between 410 and 440 nm according to the known shape of their

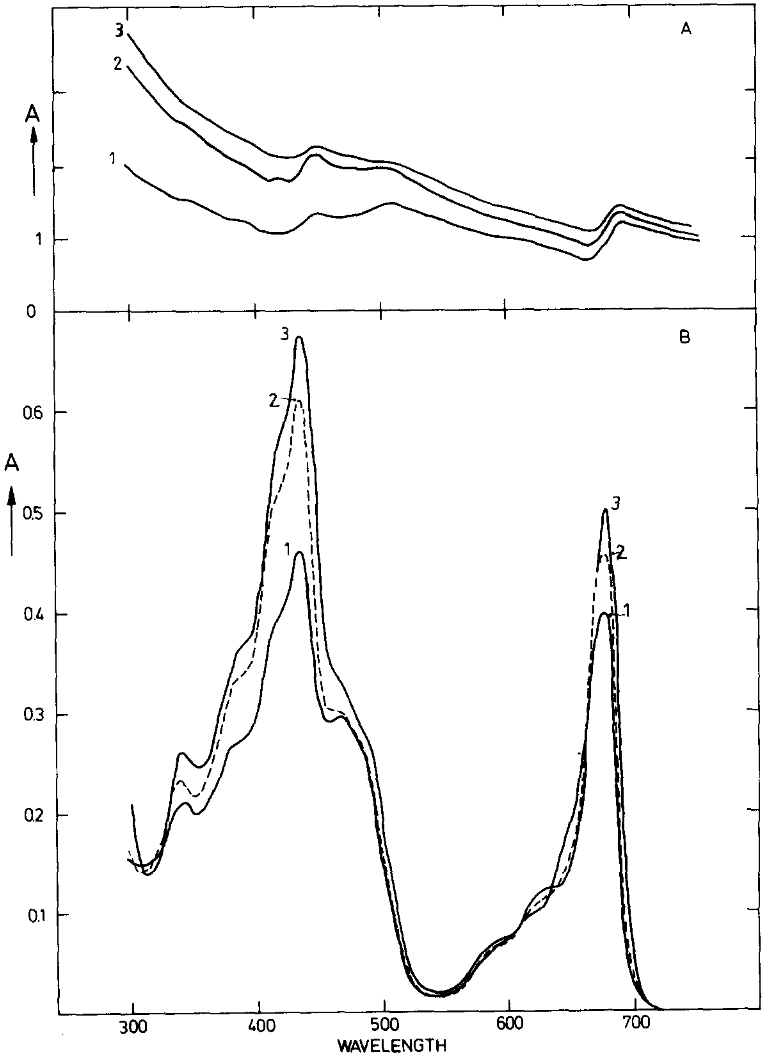

Fig. 2. Scattering spectra of chloroplast from wild type tobacco (1) and the mutants Su/su (2) and Su/su var. Aurea (3) obtained by subtraction of the absorbance spectra measured at small angle of detection from those measured at large angle of detection (A). After normalization, and assuming zero absorbance at 750 nm, scattering was subtracted from the large angle spectra to obtain the corrected absorbance spectra (B).

difference spectra which yielded the absorbance changes of cytochrome *f* at 423.5 nm and at 430 nm of P-700 (Fig. 1). At 700 nm the absorption change was practically pure P-700. Both from $\Delta A_{423.5}/\Delta A_{556}$ for cytochrome *f* and from $\Delta A_{430}/\Delta A_{700}$ for P-700 we calculated a differential flattening factor at 325 nm, using the known differential extinction coefficient [12,13], Eqns. 1, 2 and 3 and the absorbance spectra corrected for scattering (Fig. 2). The separation of cytochrome *f* and P-700 was repeated until the same value for the differential flattening factor at 325 nm (f'_{325}) was found with both components. Identical results were obtained with the two components in isolated spinach grana, where kinetic isolation of the absorbance changes of P-700 and cytochrome *f* is easy (Gorren, A.C.F. and van Gorkom, H.J., unpublished results).

c. Measurement of reaction center concentrations

The concentrations of the reaction centers were measured quantitatively by accumulation in continuous light of either the primary donor (P-700) in the case of PS I, or the primary acceptor (Q) for both types of PS II [2]. P-700 absorption changes were measured at 700 nm at —57° C. Q reduction was measured at 325 nm at room temperature, in the presence of 0.5 mM ferricyanide and 10 μM DCMU. The concentrations of P-700 and Q were determined from the measured absorbance changes after correction for the flattening effect and using differential extinction coefficients of 64 $\text{mM}^{-1} \cdot \text{cm}^{-1}$ [12] and 13 $\text{mM}^{-1} \cdot \text{cm}^{-1}$ [16], respectively. The ratio of PS II_α to PS II_β was obtained from an analysis of the fluorescence induction kinetics [5,6].

d. Quantum efficiencies

The intensities of incident light were measured with an YSI Kettering model 65 radiometer. The fraction of quanta absorbed by the chloroplasts from the broad band of light transmitted by a heat reflecting filter and Corning CS 4-96 and CS 3-67 filters was found to be about 8% for the samples of all three types of chloroplasts, as measured with a Siemens BPX 65 photodiode (Fig. 2). The light-loss at the windows of the low temperature attachment was measured with the same photodiode.

From the kinetics of P-700 oxidation at —57° C and of fluorescence induction rate constants for the utilization of light quanta in the primary reactions of the photosystems were obtained as described in Ref. 6. The reaction mixture contained 10 μM DCMU and 2 mM NH_2OH. In contrast to what is described in the reference we did not preilluminate the sample prior to the measurement of ΔA_{700} at —57° C, to prevent spill-over of light energy from closed PS II centers to PS I. The kinetics of P-700 were in all cases a monophasic exponential. Fig. 3 shows the fluorescence induction curves of the wild type and mutant chloroplasts. Treatment of the kinetics is explained in Ref. 6 and illustrated in Fig. 4. In the chloroplasts used for the experiment of Fig. 4, for instance, the concentration of Q_β was found to be 1 per 65 molecules of Chl; at an absorbed light intensity of 0.098 photons per Chl per second, a rate constant for the reduction of Q_β of 3.7 s^{-1} was found. From these data the partial quan-

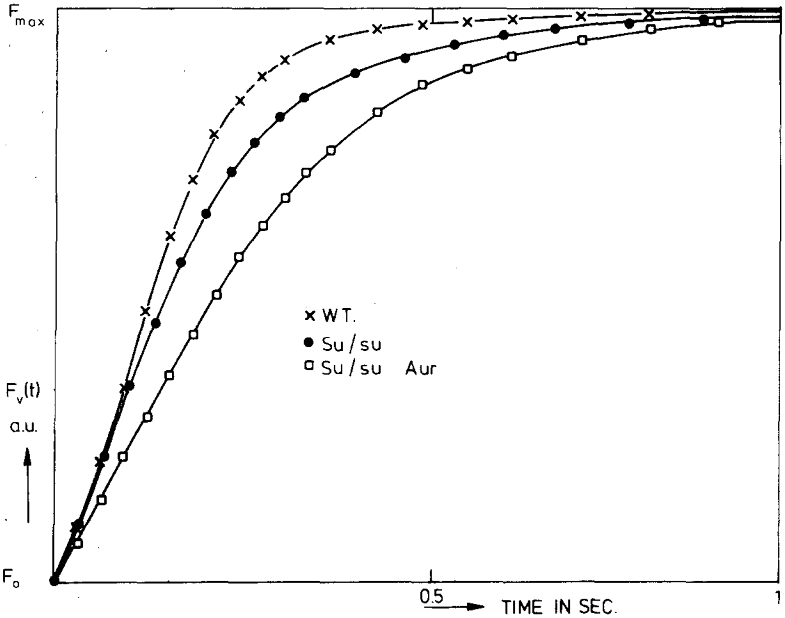

Fig. 3. Time course of the fluorescence rise in dark adapted chloroplasts of the three kinds of tobacco chloroplasts. As excitation light the light transmitted by Corning CS 4-96 and CS 3-67 filters was used. The light intensity was chosen such as to reach the maximal fluorescence level in about 1 s: for the wild type this intensity was 0.35 mW/cm^2, for Su/su 0.85 mW/cm^2 and for Su/su var. Aurea 1.15 mW/cm^2.

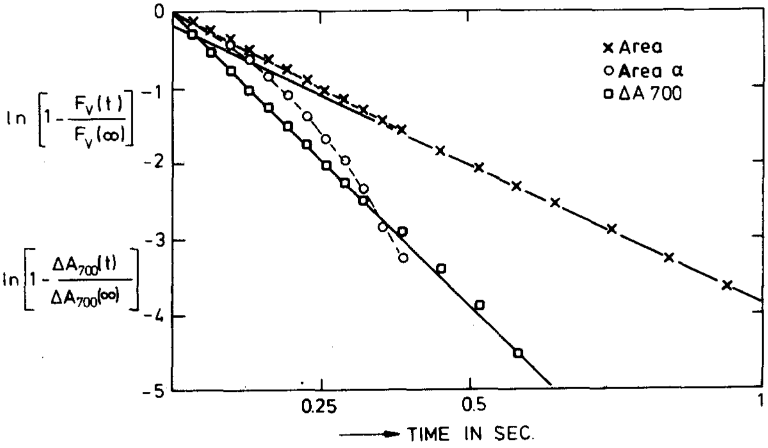

Fig. 4. Semilogarithmic plots of the rates of the primary reactions in 3.5-month-old Su/su var. Aurea (No. 2 in Table I) with the light intensity as the rate-limiting factor. The analysis of the area above the fluorescence induction curve (crosses) shows two phases. After subtraction of the slow component (k_β = 3.7 s^{-1}) the first phase clearly shows cooperativity between the units; its initial rate constant, 3.8 s^{-1}, is only slightly higher than k_β. The photooxidation of P-700 at —57°C proceeds exponentially with a single rate constant of 7.8 s^{-1} (squares). By dividing the rate constants by the number of quanta absorbed per chlorophyll molecule per second (0.098 in the fluorescence experiment and 0.088 for the oxidation of P-700), one obtains antenna cross sections of about 40 for PS II$_\alpha$ and PS II$_\beta$ and 90 for PS I.

tum yield of the photoreduction of Q_β can be calculated: $1/65(Q_\beta \cdot Chl^{-1}) \times 3.7(s^{-1})/0.098(h\nu \cdot Chl^{-1} \cdot s^{-1}) = 0.58\ Q_\beta/h\nu$.

Results

The results are summarized in Table I. With Su/su and the wild type the results did not vary significantly with the age of the plants. The figures given are mean values of six experiments. The experiments with Su/su var. Aurea were done on chloroplasts from the same batch of plants. We distinguished PS II_α from PS II_β by its sigmoidal kinetics rather than the actual rate constants, because measurements on chloroplasts preparations with different photosynthetic unit sizes had to be compared. The results demonstrate how together with increasing Chl *b* content and density of the thylakoid system, as indicated by the differential flattening factor at 325 nm, the amount of PS II_α relative to PS II_β increases, while the relative concentrations of all types of reaction centers decrease. Especially the amount of PS II_β was affected. The stoichi-

TABLE I

CHARACTERISTICS OF PHOTOSYSTEMS I, II_α AND II_β IN TOBACCO CHLOROPLASTS

Chlorophyll and reaction center composition, quantum yield and antenna sizes of PS I, PS II_α and PS II_β in chloroplasts from Su/su var. Aurea, Su/su and the wild type of tobacco. The experiments with the yellow mutant were done on the same batch of plants at intervals of about two weeks; the first experiment was done when the plants were 3 months old. The antenna sizes denote the number of Chl *a* and *b* molecules collecting energy for each of the reactions. All measurements were repeated 5 times or more. The uncertainty in the total quantum yield of Aurea chloroplasts is ±0.04 in each experiment, and ±0.02 for Su/su and wild type chloroplasts, for which average values of seven experiments are given.

Material	Su/su var. Aurea								Su/su	Wild type
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)		
$\frac{\text{Chl } a}{\text{Chl } b}$	>15	11	10	9	8	6	5.5	5	4.5	2.9
f'_{325}	0.93	0.93	0.92	0.92	0.92	0.91	0.90	0.88	0.86	0.65
$\frac{\text{P-700}}{\text{Chl}} \times 10^3$	3.4	3.3	3.1	2.9	2.9	2.5	2.5	2.6	2.5	1.9
$\frac{Q_\alpha}{\text{Chl}} \times 10^3$	<1	3.0	3.3	3.1	3.0	2.9	2.8	2.6	2.9	2.0
$\frac{Q_\beta}{\text{Chl}} \times 10^3$	16.7	15.4	13.3	13.3	11.8	10.5	8.3	8.0	5.0	1.3
System	Quantum yield									
PS I	0.32	0.30	0.29	0.27	0.27	0.25	0.28	0.32	0.32	0.35
PS II_α	—	0.12	0.17	0.19	0.24	0.26	0.30	0.33	0.38	0.48
PS II_β	0.66	0.58	0.53	0.51	0.47	0.47	0.41	0.33	0.28	0.16
Total	0.98	1.00	0.99	0.97	0.98	0.98	0.99	0.98	0.98	0.99
System	Antenna size									
PS I	90	90	95	95	95	100	110	120	125	190
PS II_α	—	40	50	60	80	90	110	125	130	240
PS II_β	40	40	40	40	40	45	50	45	55	130

ometry between the concentrations of PS I and PS II_α did not show much variation, if the first experiment on young Aurea chloroplasts is not taken into account. With respect to the quantum yield PS I is rather constant. PS II_α gains importance at the expense of PS II_β, when more and more of the Chl *b* containing light-harvesting complex is assembled.

In spite of all these changes the total quantum yield was always close to unity. Therefore we may conclude that the absorbed light intensity was distributed over the three photosystems according to their quantum yield, and that the antenna sizes of the systems were proportional to their rate constants. On the assumption that all chlorophyll species absorb about equally in the wavelength range of the actinic light, the antenna sizes were calculated from the rates of the primary reactions (cf. legend of Fig. 4). The antenna sizes are listed in Table I as the number of chlorophyll molecules collecting energy for each of the photoreactions. The figures given for the antenna size of PS II_α should be regarded as the average number of Chl molecules from the matrix which can be attributed to a unit of PS II_α.

Discussion

Our results show that, in each type of chloroplast used, essentially all quanta absorbed by chlorophyll produce a charge separation in PS II_α, PS II_β or PS I. A loss in the order of 10% must be assumed to occur due to, amongst other things, chlorophyll fluorescence and triplet formation. A small error in the extinction coefficients used or a contribution by reactions at the oxidizing side of Photosystem II to the measured changes at 325 nm [17] might explain this discrepancy. Although the PS I kinetics were measured at lower temperature than those of the Photosystems II, we checked that in each case all three reactions operated simultaneously. At −57° C energy distribution is not significantly different from that at room temperature [18]. Irrespective of the systematic errors we may conclude that the quantum efficiencies of PS II_α and PS II_β are the same, because a more than 10-fold difference in the distribution of the absorbed excitation energy between PS II_α and PS II_β in the different preparations had no effect on the total quantum yield.

It is generally accepted that PS I and PS II receive their excitation energy from different antenna systems. Our results show that essentially all chlorophyll is distributed over three types of antenna, one belonging to PS I and two belonging to PS II. We distinguished PS II_α from PS II_β by the sigmoidal kinetics of the α phase. Except in young Su/su var. Aurea mutants, and perhaps more generally at the beginning of the greening process [19], the sigmoidal phase always was more rapid (initial rate) than the exponential phase. If only one type of chloroplast is considered, the distinction between α and β centers on the basis of rate constant [1] is normally equivalent to the distinction on the basis of sigmoidicity. In the absence of Mg^{2+} the sigmoidicity of the α phase is lost, leaving two exponential phases, the rate constants of which indicate that the antenna sizes [4] and amounts (Thielen, A.P.G.M. and Melis, A., unpublished results) of the two Photosystems II are not affected by Mg^{2+}. Since the rate constants differ in different types of chloroplasts the sigmoidicity of the faster phase provided a less ambiguous criterion for our purposes.

The high total quantum yield implies that the slow rate of the β phase is due to a small antenna size and not to a low yield of PS II_β. The two different PS II antennae are associated with reaction centers which differ in other respects too: upon chemical reduction the primary electron acceptor of the β centers exhibits a higher midpoint potential than that of the α centers [3,20, 21], and its re-oxidation after photoreduction in the presence of DCMU is 10-times slower than in α centers [1]. Taken together these differences in antenna and reaction center properties seem to exclude any other explanation than the occurrence of two different, independent structures.

Because of the high quantum efficiency we could calculate the antenna sizes for each type of photosystem (see Table I). The development of the systems from young Aurea plants to the wild type resembles in some respects the development in newly synthesized chloroplasts [22]. For PS I the results suggest a basic unit of about 90 chlorophyll molecules. These are probably all Chl *a*, like in PS I particles of the same size isolated with e.g. Triton X-100 [23]. More gentle isolation procedures which leave the whole photosynthetic unit of PS I intact, yield products with 150—250 chlorophyll molecules per P-700 and with 15—20% Chl *b* [24]. This might mean that the basic unit in the wild type chloroplasts is complemented with, besides some Chl *a* bearing proteins, about 10 Chl *a*/*b* subunits.

The smallest unit observed with sigmoidal fluorescence rise kinetics (our criterion for PS II_α) had about 40 chlorophyll molecules. As has been shown by Diner and Wollmann [25] energy transfer can occur between very small units and certainly does not need the presence of the complete light-harvesting complex. In the wild type there are 225 to 250 chlorophyll molecules per center in the PS II_α antenna. PS II_β too has a minimal unit size of 40 chlorophyll molecules. Unlike PS II_α it did not grow significantly in the early stages of development. In a subsequent article it will be shown that at least up to a size of 60 chlorophyll molecules the antenna of PS II_β does not contain Chl *b*. In wild type chloroplasts its size varies between 100 and 150 chlorophyll molecules per unit.

There is a correlation between the relative abundance of α centers and the degree of membrane stacking [6], which in different plants is correlated with the average light intensity both in a genetic and in an adaptive way [26,27]. The existence of two types of PS II may allow a more efficient utilization of the wide range of light intensities to which plants are exposed.

Acknowledgements

Thanks are due to Mr. A. Wesseling for culturing the plants, and to Dr. J. Amesz for critical reading of the manuscript. This investigation was supported by the Netherlands Foundation for Chemical Research (SON), financed by the Netherlands Organization for the Advancement of Pure Research (ZWO).

References

1 Melis, A. and Homann, P.H. (1976) Photochem. Photobiol. 23, 343—350
2 Melis, A. and Duysens, L.N.M. (1979) Photochem. Photobiol. 29, 373—382

3 Horton, P. and Croze, E. (1979) Biochim. Biophys. Acta 545, 188—201
4 Melis, A. and Homann, P.H. (1978) Arch. Biochem. Biophys. 190, 523—530
5 Okabe, J., Schmid, G.H. and Straub, J. (1977) Plant Physiol. 60, 150—156
6 Melis, A. and Thielen, A.P.G.M. (1980) Biochim. Biophys. Acta 589, 275—286
7 Arnon, D.I. (1949) Plant Physiol. 24, 1—15
8 Latimer, P. and Eubanks, C.A.H. (1962) Arch. Biochem. Biophys. 98, 274—285
9 Duysens, L.N.M. (1956) Biochim. Biophys. Acta 19, 1—12
10 Amesz, J. (1964) Thesis, State University of Leiden, The Netherlands
11 Pulles, M.P.J., van Gorkom, H.J. and Verschoor, G.A.M. (1976) Biochim. Biophys. Acta 440, 98—106
12 Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160—171
13 Davenport, H.E. (1972) in Proc. 2nd Int. Congress on Photosynthesis, Stresa, 1971 (Forti, G., Avron, M. and Melandri, A., eds.), pp. 1593—1601, Junk, The Hague
14 Velthuys, B.R. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 2765—2769
15 Lach, H.J. and Böger, P. (1979) Z. Naturforsch. 32, 877—879
16 Van Gorkom, H.J. (1974) Biochim. Biophys. Acta 347, 439—442
17 Pulles, M.P.J., van Gorkom, H.J. and Willemsen, J.G. (1976) Biochim. Biophys. Acta 449, 536—540
18 Rijgersberg, C.P., Melis, A., Amesz, J. and Swager, J.A. (1979) in Chlorophyll Organization and Energy Transfer in Photosynthesis, The Ciba Foundation Symposium 61 (new series), pp. 305—322, Elsevier/North Holland, Amsterdam
19 Melis, A. and Akoyunoglou, G. (1977) Plant Physiol. 59, 1156—1160
20 Melis, A. (1975) FEBS Lett. 95, 202—206
21 Thielen, A.P.G.M. and van Gorkom, H.J. (1981) in Proc. 5th Int. Congress on Photosynthesis (Akoyunoglou, G., ed.), International Science Services, Jerusalem, in the press
22 Akoyunoglou, G. and Michelinaki-Maneta, M. (1975) in Proc. 3rd Int. Congress on Photosynthesis, 1974 (Avron, M., ed.), pp. 1885—1896, Elsevier, Amsterdam
23 Vernon, L.P., Shaw, E.R., Ogawa, T. and Raveed, D. (1971) Photochem. Photobiol. 14, 343—357
24 Anderson, J.M., Fork, D.C. and Amesz, J. (1966) Biochem. Biophys. Res. Commun. 23, 874—879
25 Diner, B.A. and Wollmann, F.A. (1979) Plant Physiol. 63, 20—25
26 Boardman, N.K. (1977) Annu. Rev. Plant Physiol. 28, 355—377
27 Wild, A. (1979) Ber. Dtsch. Bot. Ges. 92, 341—364