Ultrafast spectroscopy studies on the mechanism of electron transfer and energy conversion in the isolated pseudo ginseng, water hyacinth and spinach chloroplasts

XU Sichuan (徐四川)[1], SUN Zhaoyong (孙照勇)[1], AI Xicheng (艾希成)[1], FENG Juan (冯 娟)[1], ZHANG Qiyuan (张启元)[1], ZHANG Xingkang (张兴康)[1], YU Fei (郁 飞)[2], TANG Chongqin (唐崇钦)[2], LI Liangbi (李良璧)[2] & KUANG Tingyun (匡廷云)[2]

1. State Key Laboratory for Structural Chemistry of Unstable and Stable Species, Center of Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China;
2. Photosynthesis Research Center, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China
Correspondence should be addressed to Xu Sichuan(email: xusc@chem.pku.edu.cn)

Received February 20, 2001

Abstract The spectroscopy characteristics and the fluorescence lifetime for the chloroplasts isolated from the pseudo ginseng, water hyacinth and spinach plant leaves have been studied by absorption spectra, low temperature steady-state fluorescence spectroscopy and single photon counting measurement under the same conditions and by the same methods. The similarity of the absorption spectra for the chloroplasts at room temperature suggests that different plants can efficiently absorb light of the same wavelength. The fluorescence decays in PS II measured at the natural Q_A state for the chloroplasts have been fitted by a three-exponential kinetic model. The three fluorescence lifetimes are 30, 274 and 805 ps for the pseudo ginseng chloroplast; 138, 521 and 1494 ps for the water hyacinth chloroplast; 197, 465 and 1459 ps for the spinach chloroplast, respectively. The slow lifetime fluorescence component is assigned to a collection of associated light harvesting Chl a/b proteins, the fast lifetime component to the reaction center of PS II and the middle lifetime component to the delay fluorescence of recombination of P_{680}^{+} and $Pheo^{-}$. The excitation energy conversion efficiency (η) in PS II RC is defined and calculated on the basis of the 20 ps electron transfer time constant model, 60%, 87% and 91% for the pseudo ginseng, water hyacinth and spinach chloroplasts, respectively. This interesting result is in unconformity with what is assumed to be 100% efficiency in PS II RC. Our result in this work stands in line with the 20 ps electron transfer time constant in PS II rather sound and the water hyacinth plant grows slower than the spinach plant does as envisaged on the efficiency. But, our results predict that those plants can perform highly efficient transfer of photo-excitation energy from the light-harvesting pigment system to the reaction center (closely to 100%). The conclusion contained in this paper reveals the plant growth characteristics expressed in the primary processes of photosynthesis and a relationship between a plant growing rate and its spectroscopy characteristics and fluorescence lifetimes, namely, the slower a plant grows, the less excitation energy conversation efficiency used might be anticipated.

Keywords: pseudo ginseng, water hyacinth, chloroplast, single photon counting, fluorescence lifetime, excitation energy conversation efficiency.

In 1960, Arnold and Clayton[1] reported that the first light-chemical step in the purple bacte-

rium photosynthesis is the electric charge separation called primary reaction, which results in holding dynamics for photosynthesis. Currently, the primary processes of photosynthesis in green plants have still been an important topic of interest[2—5]. Chlorophyll (Chl) fluorescence has been used to understand excitation energy transfer processes, pigment orientation and protein organization in extensive experimental and theoretical work for almost two decades[6—9]. It is well known that the primary processes of photosynthesis in green plants have been shown to be highly efficient and energy losses constitute only minor deactivation pathways. But, there is still a finite probability that it will decay either by non-radiative process or by fluorescence before trapping can take place during excitation migration in the antenna, the separation and stabilization[9].

Green plant growth is related with or controlled by a number of factors. One of them is plant genome, an intrinsic nature that dominates green plant growth. Recently, it was reported that the genome sequences of a number of bacterial species and several eukaryotes were completed while nucleotide sequencing of the *Arabidopsis* genome is nearing completion and sequencing of the rice genome has been just finished, and a large amount of expressed sequence tag information is being obtained for many other plants[10,11]. This wealth of sequence information is used to accelerate progress toward a comprehensive understanding of the genetic mechanisms that control plant growth, development and response to the environment. Although different plant growths and developments are dominated by their genomes which determine how the plants use the energy, however, according to the law of energy conservation, the faster a plant grows, the more the energy should be preserved, and the slower a plant grows, the less the chemical energy should be stored. It depends on the efficiency of absorbing light turned to excitation energy by chlorophyll and carotene in per mass chloroplast, the translation energy efficiency and the excitation energy conversation efficiency. In other words, the difference may be expressed in the primary processes of photosynthesis.

Based on the analysis above, we adopt a few of different plant chloroplasts and compare them with their primary processes of photosynthesis to obtain more information so that the characteristics of the primary processes of photosynthesis in different green plants can be further understood. In this work, three kinds of chloroplasts isolated from pseudo ginseng (*Panax pseudo-ginseng*)[12] growing up for 3—7 a at Wenshan of Yunnan Province, and water hyacinth (*Eichhornia crassipes*)[13] growing rapidly at Dian Chi in Kunming of China and Spinach (*Spinacia oleracea* L.) purchased from local market, which are internationally extensively studied by researchers in the chemical, biological and physical fields, have been studied by absorption spectra, low temperature steady-state fluorescence spectroscopy and single photon measurement under the same conditions. We will distinguish and argue on the origin of fluorescence decay component, define and measure the excitation energy conversation efficiency in the primary processes of photosynthesis to probe the electron transfer time constant and the growth characteristics of green plant interrelated with its spectroscopy characteristics and fluorescence lifetimes.

1 Materials and methods

1.1 Isolation of chloroplast

The isolation method for the chloroplasts can be seen in refs. [14, 15]. Deveined fresh leaves washed by distilled water were homogenized by a mixer in a preparation medium buffer (pH 7.4) containing 0.2 mol/L NaCl, 0.1 mol/L sucrose, and 0.05 mol/L phosphate. After filtration through 4 and 16 layers of gauze, the green juice was centrifuged at 600 g for 5 min and the precipitate was discarded. The supernatant was again centrifuged at 1500 g for 10 min and the precipitate was suspended in a medium buffer (pH 6.9) containing 0.05 mol/L NaCl, 0.3 mol/L sucrose, and 0.05 mol/L phosphate. Preparation procedures for the chloroplasts were finished at 4℃ and in dark. The isolated samples were stored in liquid nitrogen until use.

1.2 Experimental methods

Chlorophyll protein content and Chl a/b ratio for the chloroplasts were determined according to the method in ref. [16] on UV-190 split-beam spectrophotometer. The result is listed in table 1. Among the three chloroplasts, the value of Chl a/b ratio is the biggest in the water hyacinth chloroplast and the smallest in the pseudo ginseng chloroplast, meaning that in the water hyacinth chloroplast the amount of Chl b is relatively less than Chl a, and vice versa in the pseudo ginseng chloroplast, compared with that occurring in the water hyacinth and spinach chloroplasts.

Table 1 The Chl a / b ratio for the pseudo ginseng, water hyacinth and spinach chloroplasts

	Water hyacinth	Spinach	Pseudo ginseng
Chl a/b ratio	3.36	3.03	2.43

Absorption spectra and their fourth derivative absorption spectra were recorded by UVIKON943 double-split-beam spectrophotometer under the condition of 4℃ and avoiding light[17]. Low temperature steady-state fluorescence spectroscopies were measured according to the method of Tang et al.[14] at liquid nitrogen temperature using Hitach Ltd. F-4500 fluorescence split-beam spectrophotometer. The Chl concentrations of the samples were diluted to 10 μg/mL.

Time-correlated single photon count is an experimental method to measure fluorescence decay time[18,19] with the advantages of high sensitivity, wide spectrum and weak light strength to radiate samples[20]. Time-resolved fluorescence measurements were performed using a synchronously pumped and cavity-dumped dye laser system (Spectra Physics: SP3500 Model) with a mode-locked YAG laser (Spectra Physics: SP3800 Model) as the pumping source. An intracavity acousto-optically dumped Rhodamine 6G dye laser at 570—610 nm for pulse selecting at 800 kHz, was used. The maximum laser pulse intensity (1×10^7 photons per cm^2) was attenuated with output laser power of 50 mW. A microchannel plate detector (Hamamatsu, Model R1645-01u) was applied to detecting single photon. The 10 mV anode signal from the detector was amplified with a Hewlett Packard HP8447f pre-amplifier, with amplifier combination being 0.6—1.0 V. Fluores-

cence was collected at 90° using a Ditric Optics 3-plate birefringement. The instrument response function had an FWHM of 100 ps. Total 1024 channels of the single photon with 25 ps per channel were recorded. The count is 10000 at peak. This single-counting system detects light pulses of typical 30 ps duration at variable repetition rates. The chloroplast samples all were excited at 580 nm and detected at 682 nm. The optical length of the cuvette for the measurement was 1.0 cm. The chloroplast suspension was diluted with the buffer to a final concentration of 10 μg/mL Chl, 0.4 mol/L sucrose, pH: 6.5, 50 mmol/L Mes, and 10 mmol/L NaCl. Life-times according to multiexponential kinetics were calculated by an iterative deconvolution method using a semi-linear Marqurdt-algorithm. The quality of the fits was judged by both a reduced X^2 criterion and a plot of weighted residuals. The fitting program (ASUFIT) used in this work was presented by Photosynthesis Research Center in Arizona State University.

2 Experimental results

2.1 UV absorption spectra

Fig. 1 (a) shows absorption spectra of the pseudo ginseng, water hyacinth and spinach chloroplasts, and fig. 1(b) is their fourth derivative absorption spectra at room temperature. Absorption peaks at 680 and 438 nm and shoulder peaks at 649 and 484 nm are generated by Chl a and Chl b absorbing light, respectively. Besides, carotenoid originates absorption peak at 438 nm. In the fourth derivative absorption spectra, absorption peak at 680 nm is split into two peaks at 669 and 682 nm, responsible for two different states of Chl a molecules. Absorption spectra and their fourth derivative absorption spectra both display an analogy for the pseudo ginseng, water hyacinth and spinach chloroplasts efficiently absorbing light of the same wavelength. However, fig. 1 shows that the absorption intensity at 400—500 nm for the water hyacinth chloroplast is stronger than those for the other chloroplasts. This result discovers that the same preparation ap-

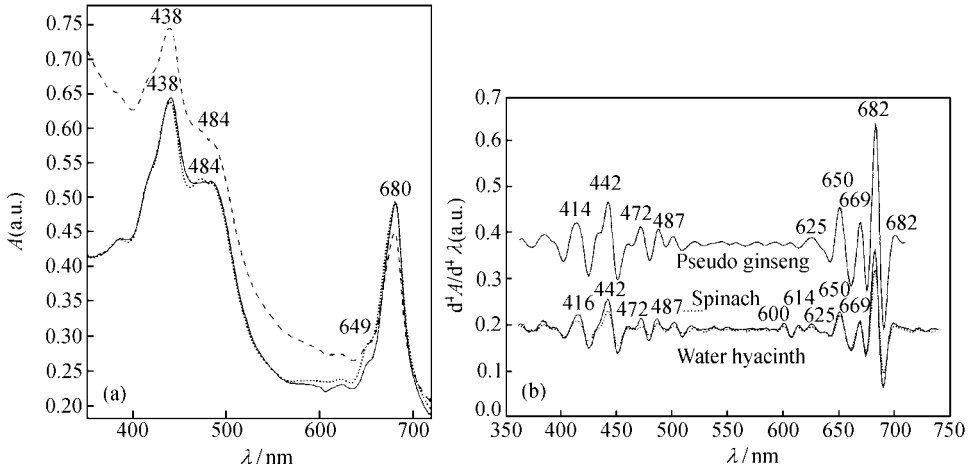

Fig. 1. Absorption spectra at room temperature for the pseudo ginseng, water hyacinth and spinach chloroplasts. (a) Absorption spectra; (b) the fourth derivative absorption spectra.

pears to remove an amount of carotenoid molecules less for the water hyacinth chloroplast clued further by the strongest absorption peak at 438 nm.

2.2 Fluorescence spectra at low temperature

Fig. 2 shows fluorescence emission spectra of the pseudo ginseng, water hyacinth and spinach chloroplasts at liquid nitrogen temperature. In the fluorescence emission spectra derived from both Chl a absorbing at 436 nm (λ_{ex} = 436 nm) and Chl b absorbing at 480 nm (λ_{ex} = 480 nm), fluorescence emission at 683 nm comes from PS II and 731 or 738 nm from PS I[21]. Only for the spinach chloroplast, can the fluorescence emission peak at 691 nm be generally reckoned to come from its PS II internal antenna. With respect to the fluorescence emission for the spinach chloroplast, the fluorescence emission in PS I for the pseudo ginseng chloroplast exhibits red shift by 10 nm. In the PS II reaction center, the electric charge separation makes oxidized and reduced species with highly energy appear. In the side of oxidized species, water is oxidized into oxygen and the reduced species connects with the PS I that possesses its own light-harvesting pigment system. In the primary processes of photosynthesis for green plant photosynthesis system, to maintain highly efficient photosynthesis rate, it is necessary to keep balance between two photo systems that photo quantum nearly equally enters into PS II and PS I[22,23]. Low temperature steady-state fluorescence spectroscopy of PS II and I may account for the balance at some extent. If F_{683}, F_{731} and F_{738} represent PS II and PS I relative fluorescence intensity for the three plant chloroplasts, the value of F_{731} / F_{683} (spinach), F_{732} / F_{682} (water hyacinth) and F_{738} / F_{683} (pseudo ginseng) (see fig. 3 (a)) can be applied to judging the distribution of excitation energy between two photo systems and estimate the balance. The values of ratios between two photo systems are 0.29， 0.37 and 0.85 for the pseudo ginseng, water hyacinth and spinach chloroplasts, respectively, which discloses that the balance for the spinach chloroplast is the best, while the balance for the pseudo ginseng chloroplast the worst. The similar values are 0.31, 0.49 and 0.90 for the pseudo ginseng, water hyacinth and spinach chloroplasts observed in fig. 3 (b) by Chl b absorbing at 480 nm. It is interesting to notice that pseudo ginseng plant growing slower is in accordance with the less balance of distribution of excitation energy between two photo systems. This result may not be an accidental coincidence, possibly an indication for the less efficient photosynthesis in the pseudo ginseng chloroplast.

The fluorescence excitation spectra of the pseudo ginseng, water hyacinth and spinach chloroplasts at 77 K are shown in fig 3. An observation is that in fig. 3 (a) the same intensity peak at 437 nm arising from Chl a as that at 483 nm from Chl b appears in the pseudo ginseng chloroplast. This result illustrates that light energy absorbed by Chl a and b in the pseudo ginseng chloroplast fabricates a similar contribution to fluorescence emission (F_{683}) in PS II. In fig. 3 (b), the less ratio of contribution to PS I fluorescence emission (F_{740}) by Chl b absorbing at 474 nm is ascertained.

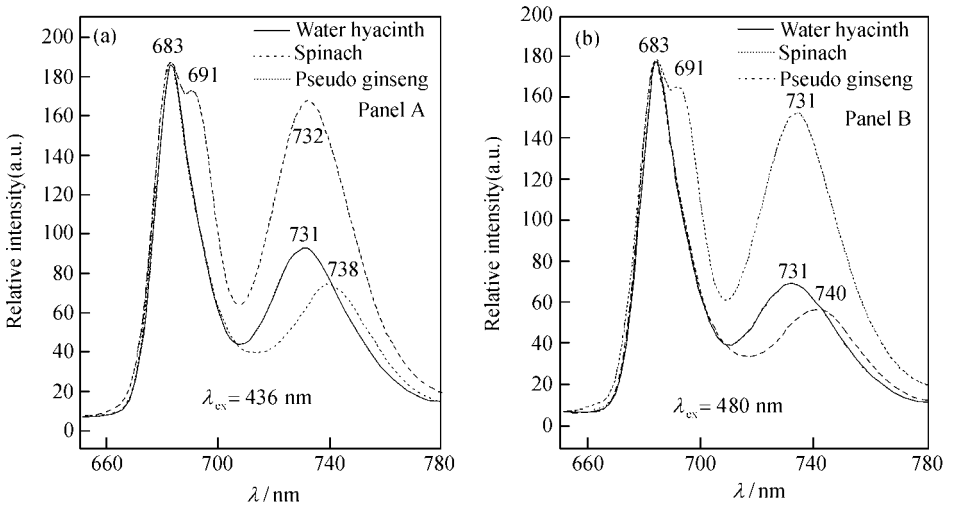

Fig. 2. Fluorescence emission spectra for the pseudo ginseng, water hyacinth and spinach chloroplasts at 77 K.

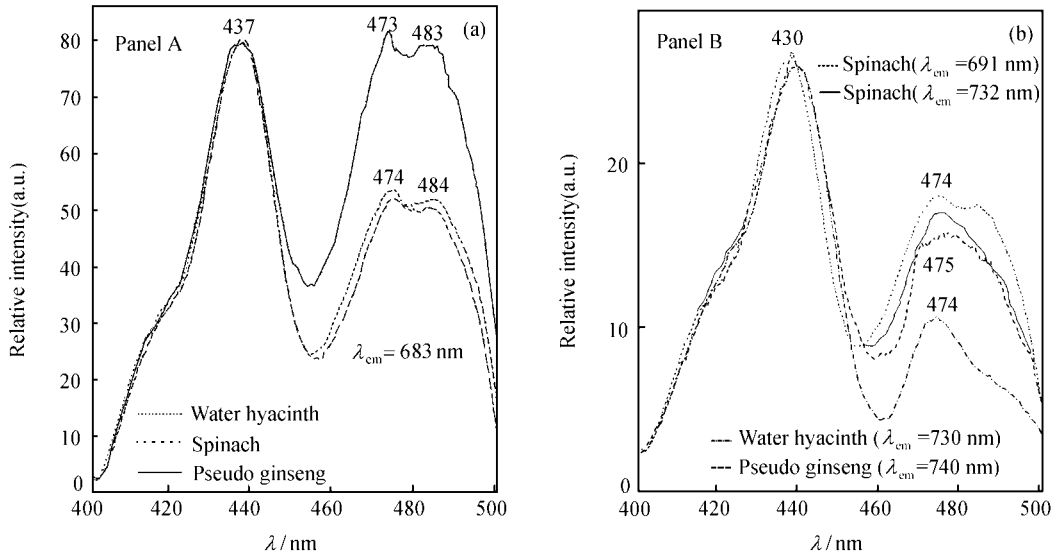

Fig. 3. Fluorescence excitation spectra for the pseudo ginseng, water hyacinth and spinach chloroplasts at 77 K.

2.3 Fluorescence lifetimes

During the primary processes of photosynthesis, the non-radiative process and the radiative process may both play an important role in the decay of excitation energy in the plant. In this work, we have taken fluorescence decay lifetimes including the two processes measured by a time-resolved chlorophyll fluorescence of the isolated samples.

The measured fluorescence decay curves in PS II at room temperature for the pseudo ginseng, water hyacinth and spinach chloroplasts are shown in fig. 4. At this point, we do not add DCMU

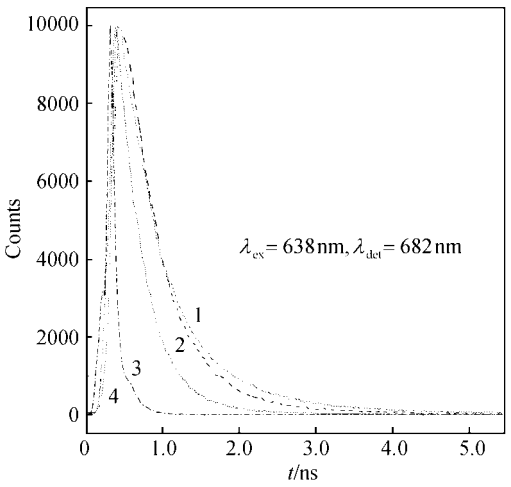

Fig. 4. Instrument response curve and fluorescence decay curves in PS II at room temperature for 1, water hyacinth; 2, spinach; 3, pseudo ginseng; and 4, laser.

or potassium ferricyanide into the samples to affect the Q_A state in order to study the near natural states in PS II of the chloroplasts for a comparison. Furthermore, we try to maintain the same experimental conditions in all stages for the different chloroplasts including Chl a/b ratio determined, absorption spectra recorded, low temperature steady-state fluorescence spectroscopy measured and time-resolved fluorescence measurements performed. It is found that the quality of the fits judged by both a reduced χ^2 criterion and a plot of weighted residuals was not improved from three kinetic components to four kinetic components so that a three-component model to fit fluorescence decay curves was used. The lifetime of slow component depends on the fitting range. In table 2, the results for a fitting range of 4.0, 4.5 and 5.0 ns are listed including their values of a reduced χ^2 criterion less than 1.5 and amplitude values. The weighted residuals are given in fig. 5. Their values responsible for the accuracy of a fitted result are all less than ± 4 in the fitting range with a uniform line.

The fitted results illustrate that among the three chloroplasts for the pseudo ginseng chloroplast the fluorescence lifetimes of three decay components in PS II are the smallest. The lifetime (τ_f) of fast fluorescence decay component in the PS II of the pseudo ginseng chloroplast is just 30 ps with the largest amplitude 60%. In the spinach chloroplast the largest amplitude is the middle lifetime component (66%, 465 ps), but in the pseudo ginseng chloroplast the largest amplitude is the fast lifetime component (60%, 30 ps), while the lifetime of fast component is 190 ps for the spinach chloroplast. It generally agrees with the origin of fluorescence decay for the slow component arising from the peripheral antenna so that it may account for that the peripheral antenna little decays absorbed

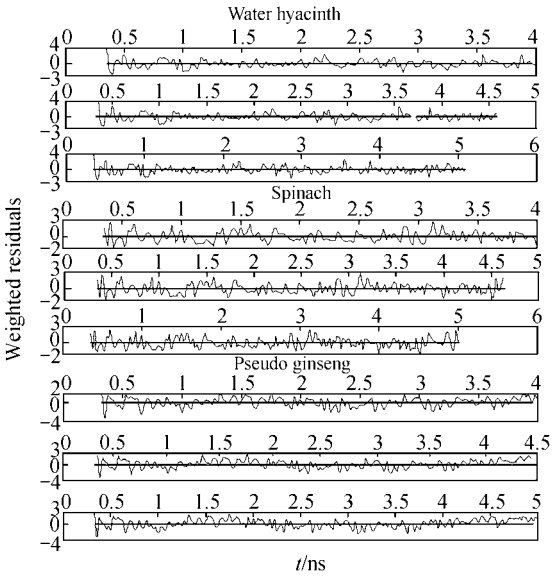

Fig. 5. The weighted residuals (all less than ±4) for the fitting ranges of 4.0, 4.5 and 5.0 ns for the pseudo ginseng, water hyacinth and spinach chloroplasts, *Y*-coordinate is for the weighted residuals and *X*-coordinate for the fitting ranges. 1, Water hyacinth; 2, spinach; 3, pseudo ginseng; 4, laser.

light energy during the primary processes of photosynthesis in green plants, because of the amplitude of the slow lifetime component being the smallest for all the chloroplasts and less contribution to fluorescence decay. It should be mentioned that the lifetime of fast component is obviously distinct, lowered to 30 ps for the pseudo ginseng chloroplast corresponding to 200 and 140 ps for the water hyacinth and spinach chloroplasts while the amplitude highlighted from 30%—40% to 60%. Detailed discussion for the origin of fluorescence component and applications will be given in the next section.

Table 2 The fitted results of fluorescence decay curves of PS II for the pseudo ginseng, water hyacinth and spinach chloroplasts

	0—4 ns			0—4.5 ns			0—5 ns		
	τ_f /ps	A (%)	χ^2	τ_f /ps	A (%)	χ^2	τ_f /ps	A (%)	χ^2
Spinach	196	30		190	28		205	32	
	465	66	1.05	455	68	1.03	477	64	1.05
	1469	4		1378	4		1530	4	
Water hyacinth	135	42		138	43		143	44	
	504	50	1.11	515	50	1.03	533	49	1.03
	1437	8		1476	7		1569	7	
Pseudo ginseng	29	59		30	60		31	60	
	265	38	1.30	274	38	1.42	282	38	1.54
	731	3		801	3		883	2	

3 Discussion

Explanation of fluorescence original for the experimentally observed complex decay kinetics in a green plant chloroplast has been a subject of intense discussion. Two models were found to be kinetically appropriate in isolated intact PS II of chloroplast of green plant. The simplest model in a rigorously defined quantitative manner, which has been used to interpret steady state fluorescence measurements, is the bipartite model[24] which involves two species, antenna chlorophyll and a reaction center, implying that the light harvesting chlorophyll a/b protein and PS II core antennae are so tightly coupled that they may be considered as one species. In this model, it ignores all details of energy transport through the antenna and treats only the coupling between antenna A and reaction center (RC or P_{680}). The reversible radical pair model[5,6,25] is based on fast (few ps) equilibration of exciton distribution between all PS II Chl and only considers the rate constants of electron transfer reactions in the reaction center[26] that is the limiting step in charge separation. The tripartite model and heterogeneous bipartite dynamics model were put up by modifying the bipartite model[24]. By changing PS II RC to closed state, or open state, or any state between them, more information can be obtained to understand the kinetics and energetics of the exciton trapping, charge separation, charge recombination, and charge stabilization processes in PS II and some of experimental measured fluorescence decays attach a description of components from PS I or PS II α/PS II β heterogeneous units[5,25] brought forward throughout different states of Chls conjoint to the reaction center in PS II with their own features of the distinct energy trans-

fer rates. However, our recent experiment can argue that fluorescence decay components of fast and middle lifetimes in PS II are independent of PS I[27]. The concept of α and β centers in terms of PS II centers of different antenna sizes located in different areas of the thylakoid membrane has recently been questioned and therefore such an interpretation shows a certain degree of uncertainty[9,28,29].

Fr□e Chl in solution has a typical lifetime around 5.0 ns[30]. In isolated LHCII the lifetime drops to 1.2 ns due to increased non-radiative decay[8]. Although there exist the controversies on the three-component or four-component theoretical models and the argument on the origin of fluorescence component in the green plant chloroplast, it generally agrees with the assignment of the slow lifetime component to a pool of free chlorophyll[6,31], as suggested by Marder and Raskin[32], which is supported by our measurements of relative variable fluorescence in PS II from the chloroplasts due to different states of chlorophyll[33]. However, the assignment of the fast and middle components is still in conflict with each other. Schatz et al.[5] reported a conclusion that at least two pigment pools contribute to the fast component. Recently, it is reported that the explanations of the reversible radical pair model are most compatible with recent experimental data on charge separation time constants[26]. The reversible radical pair model may predict that the fluorescence decay of any PS II population in the chloroplasts will be composed of two exponential components in that these systems in this paper have the characteristics of the fast and middle components. Mainly based on the two models and our experimental results, we suggest a hypothesis for the assignment of the fast and middle lifetime components, i.e., the fast lifetime component originates from the reaction center of PS II, and the middle lifetime component may be consistent with the delay fluorescence of recombination of P_{680}^{+} and $Pheo^{-}$. After the slow lifetime component has been attributed to a collection of associated light harvesting Chl a/b proteins, for the largely releasing absorbed light energy due to less efficient photosynthesis in the pseudo ginseng chloroplast, it is an appropriate suggestion that the fast lifetime component is assigned to the reaction center in PS II which is in a broad sense compatible with the result of Roelofs et al.[28], that is, the α-β heterogeneity of PS II results in two pairs of lifetimes for fast and middle components[28,29]. In PS II, the fluorescence emission peak at 691 nm of the spinach chloroplast is generally believed to come from its PS II internal antenna. Due to the reaction of recombination of P_{680}^{+} and $Pheo^{-}$, the middle fluorescence lifetime is delayed by more than 200 ps compared with the fast fluorescence lifetime[32]. Our experimental results tally with the conclusion that there exists the delayed 200 ps fluorescence lifetime. Our assignment of the fast lifetime component attributed from the reaction center of PS II, the middle lifetime component from the delayed fluorescence of recombination of P_{680}^{+} and $Pheo^{-}$ and the slow lifetime component from a c llection of associated light harvesting Chl a/b proteins not only coincides with the charge separation controlled model in PS II reaction center but also is able to interpret the current experimental observations very well.

For the fast lifetime component, its fluorescence kinetics can be described as follows:

$P_{680} + h\nu \rightarrow {}^1P_{680}^*$ (or ${}^1A^* + P_{680} \rightarrow A + {}^1P_{680}^*$) Rate: I_a ($k_a[{}^1A^*][P_{680}]$),

${}^1P_{680}^* \rightarrow P_{680} + h\nu$ $[{}^1P_{680}^*]\,k_f$,

${}^1P_{680}^* \rightarrow P_{680}$ $[{}^1P_{680}^*]\,k_{ic}$,

${}^1P_{680}^* \rightarrow {}^3P_{680}^*$ $[{}^1P_{680}^*]\,k_{isc}$,

${}^1P_{680}^* \rightarrow P_{680}^+$ $[{}^1P_{680}^*]\,k_r$,

In this model, I_a (or k_a) represents absorbed photo quantum or the transfer rate constant from any antenna chlorophyll to the P_{680}, k_f is the radiative rate constant for fluorescence emission, k_{ic} and k_{isc} represent nonradiative decay rate constant for interconversion and intersystem crossing, respectively, and k_r is charge separation rate constant. Notice that in this model the reversible energy transfer from the P_{680} to other chlorophylls and the delay fluorescence from the recombination of P_{680}^+ and $Pheo^-$ were not considered simply.

According to Cowan and Drisko[34] (assumed just by absorbing photo quantum to excite P_{680} for simplifying the equation), we can obtain

$$d[{}^1P_{680}^*]/dt = 0 = I_a - [{}^1P_{680}^*]k_f - [{}^1P_{680}^*]k_{ic} - [{}^1P_{680}^*]k_{isc} - [{}^1P_{680}^*]k_r,$$

then

$$[{}^1P_{680}^*] = I_a / (k_f + k_{ic} + k_{isc} + k_r),$$

next

$$\Phi_f = k_f / (k_f + k_{ic} + k_{isc} + k_r)$$

and

$$\tau_f = 1 / (k_f + k_{ic} + k_{isc} + k_r),$$

where τ_f is experimental fluorescence lifetime which is controlled by k_f, k_{ic}, k_{isc} and k_r, etc. When DCMU or potassium ferricyanide is added into the samples of the chloroplasts, the Q_A state of PS II changes, resulting in the alternation of the values of k_f, the values of k_{ic}, k_{isc} and k_r are increased or dropped. Therefore, it has brought about difficulty in analyzing the fluorescence component origin. In this work, it was emphasized that the fluorescence decay curves of PS II were measured at the same condition of the natural states in PS II.

It is still an unsolved controversy for the charge-separated rate constant of PS II reaction center. Two models 20 and 3 ps charge-separated rate constant to explain the controversy have been put forward[26,35—45]. Klug and his coworkers had published a series of papers in support of a ca. 20 ps effective time constant for the appearance of the charge-separated state. The conclusion was supported by the experiments reported by McCauley et al. and Freiberg et al. This effective time constant is consistent with a trap-limited model for charge separation in PS II. On the other hand, several groups had published data interpreted as supporting a ca. 3 ps time constant for the primary charge separation[41—45]. A 3 ps time constant is consistent with low-temperature hole-burning experiments and early predictions based on kinetic models and picosecond fluorescence experiments[5] and also in agreement with the well-established 2.5—3.5 ps time constant for

primary charge separation in the reaction center of purple bacteria[24]. Donovan et al.[46] tried to argue this controversy. They found the sensitivity of the observed transient absorption signals depending on the laser intensity. A variety of fast components, unobserved at low laser intensities, become prominent at higher laser intensities. At low excitation energy the signals observed are most consistent with a (20±2) ps effective time constant for charge separation. The primary processes in PS II appear too complicated for such a simplistic interpretation of the data. Notice that the different methods and measurement means were used, however, at the PS reaction center the main paths of excitation energy to be decayed are the non-radiative and radiative decay competing with the charge separation of excitation energy conversation and stabilization. Again at the moment, the excited energy transfer process for $^1P_{680}$* is neglected for some parts of effects to be offset by each other, so we pay attention to the charge separation process in PS II RC competing with the deactivation of excited energy by the non-radiative and radiative decay. To figure out the physical intention between the fluorescence lifetime and the effective time constant of the charge separation, the η is defined as efficiency converted from the excitation energy to electrochemical energy in PS II RC as follows:

$$\eta = 1/(\tau_r / (1/\tau_r + 1/\tau_f)) \times 100\%,$$

where τ_r = 20 ps for the (20 ± 2) ps model and 3 ps for the 3 ps model, and τ_f means the fast fluorescence lifetimes. The values of η for the different chloroplasts are listed in table 3.

In the pseudo ginseng chloroplast the excitation energy conversation efficiency η in PS II RC is 60% calculated on the basis of the 20 ps charge separation model, which is in good agreement with the distribution of excitation energy and the quantum balance between two photo systems and the growth nature of the pseudo ginseng plant, exposing that about a half of excited energy in the PS II of the pseudo ginseng chloroplast might be deactivated by the non-radiative process and radiative process during the charge separation. Two important factors, the distribution of absorbed energy associated with light harvesting Chl a/b proteins and excitation energy converted efficiency from PS II to PS I, affect the distribution of excitation energy and the quantum balance between two photo systems. One of two extreme cases is that the balance between two photo systems for the pseudo ginseng chloroplast is dominated by the distribution of absorbed energy associated with light harvesting Chl a/b proteins, and the effect of excitation conversion on balance is neglected or cannot be clarified. Another extreme case is that absorbed energy associated with light harvesting Chl a/b proteins accumulates in PS II and the balance between two photo systems for the pseudo ginseng chloroplast is just controlled by excitation energy conversion from PS II to PS I, in which the efficiency is estimated to be about 50% due to the reduced electrochemical energy connecting with PS I. The values of balance (0.30—0.37) for the pseudo ginseng chloroplast should be affected together by the two factors. Calculated on the 3 ps charge separation model, for the pseudo ginseng chloroplast η is 91%, which might not account for the balance of excitation energy distribution in the pseudo ginseng chloroplast and not accord with the nature of the plant

according to the view of energetics, although there is a plentiful Chl b of PS II in the pseudo ginseng chloroplast. In the water hyacinth chloroplast, the absorbed energy associated with light harvesting Chl a/b proteins in PS I is highlighted by accumulating Chl a in PS I and the excitation energy conversation efficiencies both are 98% in the PS II of the water hyacinth and spinach chloroplasts calculated on the 3 ps charge separation model. This result could hardly interpret that the balance of excitation energy distribution between two photo systems in the water hyacinth chloroplast is different from that in the spinach chloroplast. Throughout the excitation energy conversion efficiency and the application of the charge separation time constant model, our experimental results prove reasonable to the 20 ps effective charge separation time constant established or to a step about an effective 20 ps time in multi-steps charge separation processes in PS II. Because the excitation energy conversion efficiency in the water hyacinth chloroplast is smaller, our experimental results demonstrate that the growth rate for the water hyacinth plan is smaller than that for the spinach plant. But, due to a plentiful amount of carotenoid molecules in the water hyacinth chloroplast, the capacity of absorbing and trapping light is improved. The biggest value of η is 91% in PS II RC of the spinach chloroplast, which is clearly distinct from what is always assumed to have 100% excitation energy conversion efficiency in PS II RC. As the same argument as mentioned above, we may obtain an estimate that plants can perform highly efficient transfer of photo-excitation energy from the light-harvesting pigment system to the reaction center (near 100%) due to two factors, the slow fluorescence lifetime and fast excited energy transfer.

Table 3 Excitation energy conversation efficiency [$\eta = 1/(\tau_r(1/\tau_r + 1/\tau_f)) \times 100\%$] in PS II RC for the pseudo ginseng, water hyacinth and spinach chloroplasts

τ_r/ ps	Pseudo ginseng			Water hyacinth			Spinach		
	0—4.0 ns	0—4.5 ns	0—5.0 ns	0—4.0 ns	0—4.5 ns	0—5.0 ns	0—4.0 ns	0—4.5 ns	0—5.0 ns
20	59.2	60.0	60.8	87.1	87.3	87.7	90.7	90.5	91.1
3	90.6	90.9	91.2	97.8	97.9	97.9	98.5	98.4	98.6

4 Conclusions

Having studied the absorption spectra, low temperature steady-state fluorescence spectroscopy and the fluorescence lifetime for the isolated chloroplasts, we can get several important suggestions: (i) These plants all may efficiently absorb light photon. (ii) The fluorescence decays in PS II measured at the natural Q_A states for the chloroplasts have been analyzed by a three-exponential kinetic model and a definite assignment of fluorescence components. The slow lifetime fluorescence component is assigned to a collection of associated light harvesting Chl a/b proteins, the fast lifetime component is attributed to the reaction center of PS II and the middle lifetime component may be in agreement with the delay fluorescence of recombination of P_{680}^+ and $Pheo^-$. Such an assignment of fluorescence component in this paper has taken on comparability with the actual models but cherishes more distinct physical significance. (iii) The excitation energy conversation efficiency in PS II RC is defined and measured. The value of η calculated on

the same charge separation model is varied for the different chloroplasts. On the 20 ps model, the values of η are 60%, 87% and 91% for the pseudo ginseng, water hyacinth and spinach chloroplasts, respectively. This interesting result is not in conformity to what is assumed that the efficiency is 100% in PS II RC. But, our results show that those plants can perform highly efficient transfer of photo-excitation energy from the light-harvesting pigment system to the reaction center. From the application of the effective charge separation time constants, our results may testify the (20 ± 2) ps model with more rationality, and yet are not likely to obviate the 3 ps effective charge separation time constant. (iv) The nature of green plant growth may be expressed in the primary processes of photosynthesis. In the pseudo ginseng chloroplast isolated using the same preparation as the spinach chloroplast, there is not only a red shift about 10 nm for the fluorescence emission coming from PS I, but also a discordant balance of excitation energy distribution between two photo systems. Furthermore, the excitation energy conversation efficiency in PS II RC calculated on the 20 ps charge separation time constant for the pseudo ginseng is 60%, which falls in with the nature of the plant. Our experimental results may imply a relationship between a green plant growth and its fluorescence characteristics and fluorescence lifetime. To maintain highly efficient photosynthesis, it is necessary for green plant to contain a proportional photo quantum balance between two photo systems, efficient transfer of photo-excitation energy from the light-harvesting pigment system to the reaction centers and efficient primary charge separation with high excitation energy conversation efficiency. The slower the plant grows, the less the excitation energy conversation efficiency is satisfied.

Acknowledgements The authors thank Prof. Xia Zongju in Peking University and Prof. Peng Hangcheng in the Institute of Biophysics of Chinese Academy of Sciences for their help in carrying out the single photon counting experiment, and Dr. Lin Su in Photosynthesis Research Center of Arizona State University for presenting the fitting program (ASUFIT) initially edited by Joel Hindorff and Neal Woodbury. This work was supported by the State Key Basis Research and Development Plan (Grant No. G1998010100), National Natural Science Foundation of China (Grant Nos. 29733100, 39890390) and Postdoctoral Fund.

References

1. Arnold, W., Clayton, P. K., The first step in photosynthesis: Evidence for its electronic nature, Proc. Natl. Acad. Sci. USA, 1960, 46: 769—776.
2. Keuper, H. J. K., Sauer, K., Effect of photosystem II reaction center closure on nanosecond fluorescence relaxation kinetics, Photosynthesis Research, 1989, 20: 85—103.
3. Hodges, M., Moya, I., Time-resolved chlorophyll fluorescence studies on pigment-protein complexes from photosynthetic membranes, Biochim. Biophy. Acta, 1988, 935: 41—52.
4. Butler, W. L., Chlorophyll Organization and Energy Transfer in Photosynthesis, in Ciba Foundation Symposium (ed. Excerpta Medica), Amsterdam: Elsevier/North-Holland, 1979, 237—256.
5. Schatz, G. A., Brock, H., Holzwarth, A. R., Kinetic and energetic model for the primary processes in photosystem II, Biophy. J., 1988, 54: 397—405.
6. Gulotty, R. J., Mets, L., Alberte, R. S. et al., Picosecond fluorescence study of photosynthetic mutants of chlamydomonas reinhardtii: origin of the fluorescence decay kinetics of chloroplasts, Photochem. Photobiol., 1985, 41: 487—496.
7. Breton, J., Vermélio, A., Energy Conversion by Plants and Bacteria, Photosynthesis, Vol. 1, New York: Academic Press, 1983, 153—194.
8. Briantais, J. M., Vernotte, C., Krause, G. H. et al., Light Emission by Plants and Bacteria, New York: Academic Press,

1986, 539—586.

9. Myśliwa-Kurdziel, B., Barthèlemy, X., Strzalka, K. et al., The early stage of photosystem II assembly monitored by measyrements of fluorescence lifetime, fluorescence induction and isoelectric focusing of chlorophyll-proteins in barley etiochloroplasts, Plant Cell Physiol., 1997, 38(1): 1187—1196.
10. Cole, S. T., Brosch, R., Parkhill, J. et al., Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence, Nature, 1998, 393: 537—544.
11. Somerville, C., Somerville, S., Plant functional genomics, Science, 1999, 285: 380—383.
12. Yunnan Botany Institute, Yunnan Plant Annals (II) (in Chinese), Beijing: Science Press, 1979, 509—515.
13. Kunming Botany Institute of the Chinese Academy of Sciences, Yunnan Plant Annals (V) (in Chinese), Beijing: Science Press, 1991, 708—709.
14. Tang, Z. Q., Lin, S. Q., Zhang, Q. D. et al., Studies on photosynthetic characteristics of chloroplasts from lotus during germination, Acta Phytophysiologica Sinica, 1983, 9: 275—282.
15. Anderson, J. M., Boardman, N. K., Fractionation of the photochemical systems of photosynthesis, I. Chlorophyll contents and photochemical activities of particles isolated from spinach chloroplasts, Biochim. Biophys. Acta, 1966, 112: 403—421.
16. Arnon, D. I., Copper enzymes in isolated chloroplast, Polyphenol oxidase in beta dulgaris, Plant Physiol., 1949, 24: 1—55.
17. Droppa, M., Horváth, G., Mustárdy, L. A. et al., Structural and functional stability of isolated intact chloroplasts, Photosynthesis Research, 1981, 2: 31—38.
18. Connor, D. V., Phillips, D., Time-Correlated Single Photon Counting, London: Academic Press Inc., 1984.
19. Ware, W. R., Time-Resolved Fluorescence Spectrospy in Biochemistry and Biology, New York: Plenum Press, 1983, 23.
20. Tang, Y. Q., Xu, G. Z., Zhang, X. K. et al., Experimental Methods to Measure Structures of Active Intermediate Species, Kunning: Yunnan Science and Technology Press, 1990, 53—107.
21. Papagcorgiou, G., Chlorophyll fluorescence: An intrinsic probe of photosynthesis, in Bioenergetics of Potosynthesis (ed. Govindjee), New York: Academic Press, 1975, 319—371.
22. Zhang, Q. D., Kuang, T. Y., Effects of light intensity during growth on spectral characteristics of wheat seedlings, Acta Biophysica Sinica, 1988, 4(2): 182—187.
23. Zhang, Q. D., Effects of additional factors on fluorescent characteristics of chloroplasts from wheat seedlings grown under different light intensities, Acta Biophysica Sinica, 1988, 4(3): 192—196.
24. Berens, S. J., Scheele, J., Butler, W. L. et al., Kinetic modeling of time-resolved, Fluorescence in spinach chloroplasts, Photochem. Photobiol., 1985, 42: 59—68.
25. Vass, I., Gatzen, G., Holzwarth, A. R., Picosecond time-resolved fluorescence studies on photoinhibition and double reduction of Q_A in Photosystem, II, Biochimica Biophysical Acta, 1993, 1183: 388—396.
26. Klug, D. R., Joseph, D. M., Barber, J. et al., Primary processes in isolated photosystem II reaction centers probed by magic angle transient absorption spectroscopy, Chem. Phys., 1995, 194: 433—442.
27. Xu, S. C., Ai, X. C., Sun, Z. Y. et al., Ultrafast spectroscopy studies of the isolated PS II particles from pseudo ginseng, water hyacinth and spinach leaves, Chin. J. Chem., 2001, 59(6): 937—944.
28. Roelofs, T. A., Lee, C. H., Holzwarth, A., Global target analysis of picosecond chlorophyll fluorescence kinetics from pea chloroplasts, Biophys. J., 1992, 61: 1147—1163.
29. Melis, A., Anderson, J. M., Structural and functional organization of the photosystem in spinach chloroplasts, antenna size, relative electron transport capacity and chlorophyll composition, Biochim. Biophys. Acta, 1983, 724: 473—484.
30. Seely, G. R., Connolly, J. S., Fluorescence of photosynthetic pigments in vitro, in Light Emission by Plants and Bacteria, (eds. Govindjee, Amesz, J., Fork, D. S.), Orlando: Academic Press, 1986, 99—133.
31. Nordlund, T. H., Knox, H., Lifetime of fluorescence from light-harvesting chlorophyll a/b proteins, Excitation intensity dependence, Biophys. J., 1981, 36: 193—201.
32. Marder, J. B., Raskin, V. I., The assembly of chlorophyll into pigment-protein complexes, Photosynthetica, 1993, 28: 243—248.

33. Hodges, M., Moya, I., Modification of room-temperature picosecond chlorophyll fluorescence kinetics in photosystem-II-enriched particles by photochemistry, Biochim. Biophys. Acta, 1987, 892: 42—47.
34. Cowan, D. C., Drisko, R. L., Elements of Organic Photochemistry, Beijing: Science and Technology Press, 1976.
35. Durrant, J. R., Hastings, G. R., Hong, Q. et al., Determination of P680 single state lifetimes in photosystem two reaction centers, Chem. Phys. Lett., 1992, 188: 54—60.
36. Hastings, G. R., Durrant, J. R., Barber, J. et al., Observation of pheophytin reduction in photosystem two reaction centers using femtosecond transient absorption spectroscopy, Biochemistry, 1992, 31: 7638—7647.
37. Durrant, J. R., Hastings, G. R., Joseph, D. M. et al., Subpicosecond equilibration of excitation energy in isolated photosystem II reaction centers, Proc. Natl. Acad. Sci. USA, 1992, 89: 11632—11636.
38. Durrant, J. R., Hastings, G. R., Joseph, D. M. et al., Rate of oxidation of P680 in isolated photosystem 2 reaction centers monitored by loss of chlorophyll stimulated emission, Biochemistry, 1993, 32: 8259—8267.
39. McCauley, S. W., Baronavski, A. P., Rice, J. K. et al., A search for subpicosecond absorption components in photosystem II reaction centers, Chem. Phys. Lett., 1992, 198: 437—442.
40. Freiberg, A., Timpmann, K., Moskalenko, A. A. et al., Pico- and nanosecond fluorescence kinetics of photosystem II reaction center and its complex with CP47 antenna, Biochim. Biophys. Acta, 1994, 1184: 45—53.
41. Roelofs, T. A., Kwa, S. L. S., van Grondelle, R. et al., Primary processes and structure of the photosystem II reaction center: II. Low-temperature picosecond fluorescence kinetics of a D_1-D_2-cyt-b-559 reaction center complex isolated by short Triton exposure, Biochim. Biophys. Acta, 1993, 1143: 147—157.
42. Schelvis, J. P. M., van Noort, P. I., Aartsma, T. J. et al., Energy transfer, charge separation and pigment arrangement in the reaction center of Photosystem II, Biochim. Biophys. Acta, 1994, 1184: 242—250.
43. Holzwarth, A., Müller, M. G., Gatzen, G. et al., Ultrafast spectroscopy of the primary electron and energy transfer processes in the reaction center of photosystem II, J. Lumin., 1994, 60&61: 497—502.
44. Wasielewski, M. R., Johnson, D. G., Seibert, M. et al., Determination of the primary charge separation rate in isolated photosystem II reaction center with 500-fs time resolution, Proc. Natl. Acad. Sci. USA, 1989, 86: 524—528.
45. Wasielewski, M. R., Johnson, D. G., Govindjee, et al., Determination of the primary charge separation rate in photosystem II reaction center at 15 K, Photosynth. Res., 1989, 22: 89—99.
46. Donovan, B., Walker, II L. A., Yocum, C. F. et al., Transient absorption studies of the primary charge separation in photosystem II, J. Phys. Chem., 1996, 100: 1945—1949.