Characteristics of β-Car Pigments in Isolated Photosystem Ⅱ Reaction Center Studied by Selective Excitation*

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Abstract Fluorescence spectroscopy was used to learn the characteristics of pigments of photosystem Ⅱ. The purified photosystem Ⅱ (PS Ⅱ) reaction center contains approximately six molecules of chlorophyll *a* (Chl *a*), two β-carotene (β-Car) and two pheophytin *a* (Pheo *a*). The fluorescence spectra demonstrated that the reaction center has two different spectral form β-Car molecules designated as Car489 and Car507. Car489 is located close to Chl *a* form with peaks at 672 nm and 677 nm (Chl *a*672, Chl *a* 677) on protein D2. The function of Car489 is to protect reaction center from photodamage. Car507 is located close to Chl a forms with 672 nm (Chl *a*672) on protein D1 and can transfer energy to Chl *a*672 and/or other pigments, then to P680 to generate charge separation. A global analysis-fitting program was used to fit the fluorescence spectra. The results showed that Car489 transfers energy to Chl *a*672, then to Chl *a*677 in 61 ps, the excited state of Chl *a*677 decay to the ground state in 3 ns, on protein D2. Car507 transfers energy to P680 via some pigments in 274 ps, on protein D1. The recombinations of primary pair $P680^+Pheo\ a^-$ take place in 3.8 ns and 16 ns.

Keywords Photosystem Ⅱ; Reaction center; β-Car; Charge recombination; Energy transfer

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0 Introduction

Photosystem Ⅱ reaction center (D1-D2-Cyt b-559) complex is the smallest unit in photosystem Ⅱ that shows photochemical activity. It consists of the D1 and D2 proteins[1]. Sequence homology between the D1 and D2 subunits and the L and M subunits of purple bacteria, suggested that the D1/D2 and L/M heterodimers are likely to have similar dimensions in the membrane plane[1-5]. But they differ from each other in the numbers of pigments contained. The purple bacterial reaction center has four molecules of bacteriochlorophyll, one carotenoid and two bacteriopheophytin. On the other hand, PS Ⅱ reaction center contains six molecules of Chl *a*, two Pheo *a* and one or two β-Car[3].

There is debate on the number of pigment molecules associated with the PS Ⅱ reaction center[3,6~9]. The localization and the function of the molecules of Chl *a* and β-Car are also unclear at present. It is generally assumed that PS Ⅱ reaction center contains two β-Car molecules with different spectral form[10].

In this paper, we analyzed the fluorescence spectra of PS Ⅱ reaction center excited by 489 nm and 507 nm laser pulses to discuss the localization and function of β-Car molecules, and energy transfer between pigments.

1 Materials and methods

1.1 Materials

The liquid sample, PS Ⅱ reaction center was purified from spinach by Laboratory of Photosynthesis Basic Research, Institute of Botany, Chinese Academy of Sciences and stored in liquid nitrogen to keep its photochemical activity. Before experiment the sample was taken out and put into operation box filled with nitrogen gas. After thawing, the sample was diluted to 30 μg/ml chlorophyll concentration, then injected into sample cell. All of these operations were performed in operation box filled with nitrogen gas and in the dark.

1.2 Methods

A fluorescence spectroscopy was used. The output of a Ti:Sapphire oscillator was amplified by means of regenerated pulse amplification (Spitfire, Spectra physics), generating 1 kHz, 800 nm, 100 fs pulses. This light was used for driving an optical parametric amplifier (OPA, spectra physics), tunable in the

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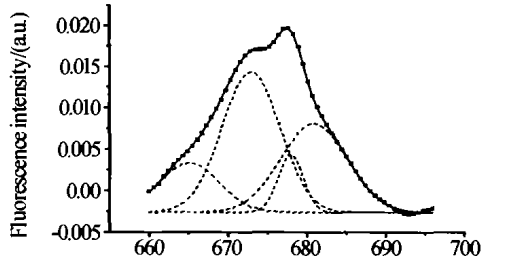
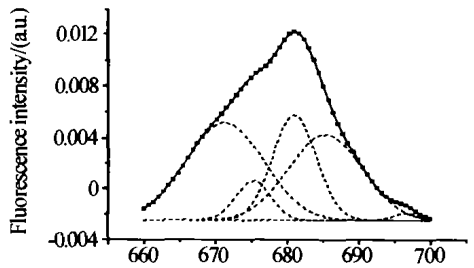
visible and infrared. In the present study, the wavelength was tuned at 489 nm and 507 nm respectively . The energy of single pulse was about 300 nJ/pulse. The sample cells were putted in a constant temperature pool cooled by semiconductor. In this study, the temperature were controlled at 20℃. The fluorescence emission after exciting was collected by a self-made fluorescence collector with collecting efficiency 30% , then was coupled to a spectrograph. Last the single color fluorescence was detected and collected by a PMT and a Boxcar 4400 Signal Averaging Processor.

2 Results and discussion

Some reports suggested the presence of two spectral forms of β-Car, i. e. the short-wavelength form with absorption peaks at 429 nm,458 nm,489 nm, the long-wavelength form with peaks at 443 nm,473 nm, and 507 nm[10~12]. In this study, the excitation light was tuned at 489 nm and 507 nm, respectively. With the two different wavelength excitation, PS Ⅱ reaction center gave different fluorescence spectra as shown in Fig. 1(a),(b). In the case of the 489 nm excitation, the fluorescence spectrum showed a peak at 678 nm and a shoulder at 673 nm, and in the case of the 507 nm excitation, the peak red shifted to 681 nm and the shoulder red shifted to 675 nm. The different spectra indicated that only one β-Car molecule was selectively excited upon the two different excitation 489 nm and 507 nm, respectively. Also Gaussian bands were used to fit the spectra of PS Ⅱ reaction center. The fits showed four bands at 665 nm,673 nm,678 nm and 681 nm in the case of 489 nm, and five bands at 671nm,675 nm, 681 nm,685 nm and 697 nm in the case of 507 nm. Fig. 2 showed the second derivative spectra. In the case of 489 nm, four components 662 nm,672 nm,678 nm, and 690 nm were shown, and in the case of 507 nm, five components 663nm,667 nm,675 nm,681 nm and 697 nm were shown. All these results showed that, at least two fluorescence spectral components,678 nm and 673 nm, were generated upon excitation 489 nm, and 681 nm,675 nm upon excitation 507 nm. Also it should be noticed that there is emission band at 697 nm at excitation 507 nm. The differences of spectra at excitation 489 nm and 507 nm also demonstrated that the two β-Car of PS Ⅱ reaction center have different absorption, localization and function. The β-Car excited selectively by 489 nm excitation was designated as Car489, and the other β-Car excited by 507 nm was designated as Car507.

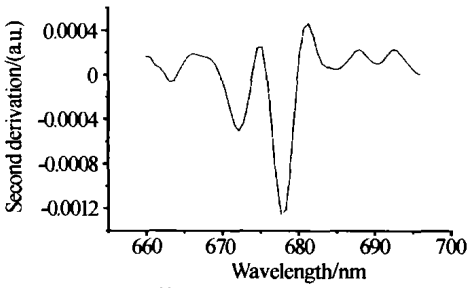


(a)Excited by 489nm laser,the largest peak is at 678nm. Gaussian bands are 665nm,673nm,678nm and 681nm

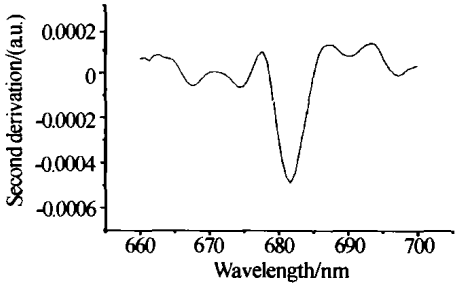


(b)Excited by 507nm laser,the largest peak is at 678nm. Gaussian bands are 671nm,675nm,681nm,685nm and 697nm

Fig. 1 The fluorescence spectra (square symbols) and fits (solid lines) with Gaussian bands (dash lines) of PS Ⅱ reaction center



(a)Excited by 489nm laser,four components 662nm, 672nm,678nm and 690nm were shown



(b)Excited by 507nm laser,fove components 663nm, 667nm,675nm,681nm and 697nm were shown

Fig. 2 The second derivative spectra of PS Ⅱ reaction center

The absorption spectrum of PS Ⅱ reaction center with a peak at 674.5 nm was shown in Fig. 3. Fit of Gaussian bands showed there are three molecules of Chl *a* absorbing at 667 nm, 672 nm and 680 nm (designated as Chl *a*667, Chl *a* 672, Chl *a*680) in reaction center. So we thought that the emission at 673 nm upon excitation 489 nm and the emission at 675 nm upon excitation 507 nm were due to Chl *a*672, and the emission at 681 nm upon excitation 507 nm was due to Chl *a*680 which had photochemical activity. The fluorescence spectra (Fig. 1) didn't clearly show the emission of Chl *a*667, so the Chl *a*667 molecules can

be established to function as antannas. The group of Kimiyuki Satoh ever extracted one Chl *a* absorbing at 677 nm with 30 ~ 50% water-saturated treatment[10]. The emission at 678 nm upon excitation 489 nm maybe due to Chl *a* absorbing at 677 nm that was not resolved in absorption spectrum (Fig. 3). The bandwidth of emission at 678 nm was only about 3 nm, which means Chl *a*677 is a monomer.

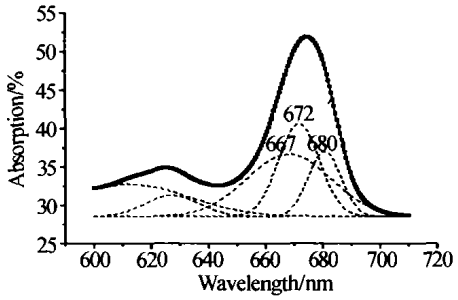


Fig. 3 The absorption spectum (square symbols) and fit (solid line) of Gaussian bands (dash lines) of PS Ⅱ reaction center. The largest peak is at 764.5 nm. There are three Gaussian bands 667 nm, 672 nm and 680 nm in long wavelength region

The emission bands 678 nm and 673 nm in the case of 489 nm indicated that the Car489 is in close proximity to Chl *a*672 and Chl *a*677, not in close to photochemical reaction pigment P680. Whereas the emission bands 681 nm and 675 nm in the case of the 507 nm indicated that Car507 is in close to Chl *a*672, and can transfer energy to P680 via other pigments. PS Ⅱ reaction center consists of the D1 and D2 proteins. It is generally accepted that D1 protein contains the most of active components. So Car489, Chl *a*672 and Chl *a*677 may be located on D2 protein, and Car507 may be located on D1 protein. This point was different from reference [10], which suggested Car489 and Car 507 locat on protein D1 and D2, respectively.

In PS Ⅱ reaction center preparation, the plastoquinones QA and QB were removed from their binding sites, implying that photochemistry does not proceed beyond the primary radical pair. So the primary radical pair $P680^+$ Pheo a^- generated by charge separation was easy to recombine. The recombination of $P680^+$ Pheo a^- can be written as

$$P680^+ \cdot \text{Pheo } a^- \rightarrow P680^* \cdot \text{Pheo } a \quad (1)$$

$$P680^* \cdot \text{Pheo } a \rightarrow P680 \cdot \text{Pheo } a + h\nu \quad (2)$$

$$P680^+ \cdot \text{Pheo } a^- \rightarrow {}^3P680 \cdot \text{Pheo } a + \text{heat} \quad (3)$$

Where $P680^*$ is excited singlet P680. 3P680 is excited triplet P680. (1) and (2) showed $P680^+$ Pheo a^- recombined to excited singlet P680 then emitting fluorescence. (3) showed $P680^+$ Pheo a^- recombined to excited triplet P680. This was also suggested by James Barber[1], to have 30% yield at room temperature.

The main functions of carotenoid in photosynthetic systems are (1) to absorb light and transfer it to other pigments (chlorphylls in plants, algae and cyanobacteria and bacteriochlorophylls in photosynthetic bacteria), which then deliver the energy to the photochemical reaction center, (2) to protect the organism from harmful (bacterio) chlorophyll triplet states that may give rise to the poisonous singlet oxygen and from singlet oxygen[1,13~16]. In PS Ⅱ reaction center, the key role of the two β-Car molecules is to protect reaction center from strong light. James Barber suggested that β-Car molecule is not very close to P680[1], so $^3P680$ can not be quenched to ground state by β-Car, and mostly reacts with molecular oxygen to generate singlet oxygen which is poisonous to reaction center. β-Car molecule may protect reaction center by quenching the singlet oxygen.

The fluorescence spectra were fitted with a global analysis-fitting program. In the case of excitation 489 nm, the decays at emission wavelength 670 ~ 690 nm were recorded, and three lifetime components were needed to describe the whole data. The lifetimes were 61 ps, 3 ns and 10 ns. Fig. 4 showed the fitting results. The dotted line was experiment data. The solid line was fitting curve. The corresponding DASs (decay associated spectrum) were shown in Fig. 5(a). The DAS of the fastest lifetime of 61 ps showed two bands around 677 nm and 684 nm. It probably reflected an energy transfer process. The second lifetime 3 ns component having a DAS peak at around 678 nm was caused preferentially by Chl *a* molecule that are energetically decoupled from the reaction center photochemistry. The longest lifetime 10 ns component having a DAS peak at around 678 nm may be due to the process of Chl *a*677 emitting at 678 nm delays to triplet state ^{3}Chl *a*677 by intercrossing. ^{3}Chl *a*677 can be decayed to ground state by Car489.

In the case of excitation 507 nm, the decays at emission wavelength 670 ~ 690 nm were also recorded, and three lifetime components were also needed to describe the whole data. The lifetimes were 274 ps, 3.8 ns and 16 ns. The corresponding DASs were shown in Fig. 5(b). The DASs of the three components showed almost same peak at around 682 nm. Many studies suggested the charge separation of PS Ⅱ reaction center takes place in about 3 ps or 20 ps[6,17~19]. In this study, such short lifetime component was not found. The fastest lifetime 274 ps component was due to the process that Car507 transfers energy to P680 via other Chl*a* molecules. The long lifetime 3.8 ns and 16 ns components were originated from recombination of

primary pair $P680^+$ Pheo a^- by two ways of (1) and (2), and (3).

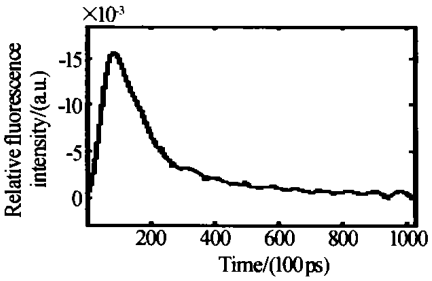
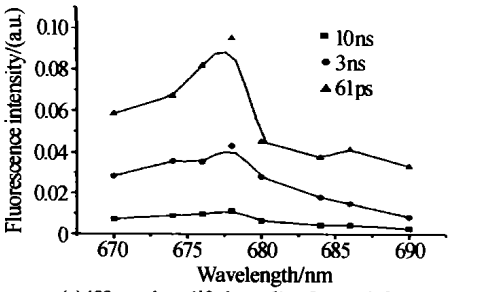
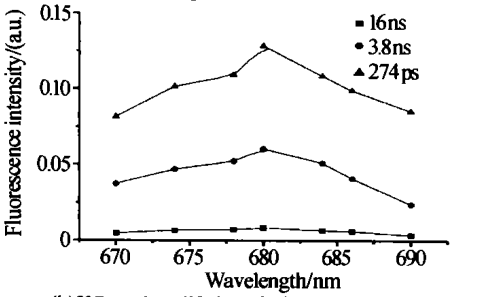


Fig. 4 The fitting results of 682 nm fluorescence of photosystem Ⅱ reaction center. (The dotted line: experiment data, the solid line: fitting curve)



(a)489 nm, three lifetimes 61 ps, 3 ns and 10 ns were needed for a good fit



(b)507 nm, three lifetimes 274 ps, 3.8 ns and 16 ns were needed for a good fit

Fig. 5 The DASs of PS Ⅱ reaction center in the case of excitation 489 nm and 507 nm

3 Conlusions

The above results demonstrated that PS Ⅱ reaction center has two different spectral form β-Car molecules designated as Car489 and Car507. Car 489 is located on protein D2 and close to Chl *a*672 and Chl *a*677, whereas Car 507 is located on protein D1 and close to Chl *a*672 which is in a position symmetrical to that of Chl *a*672 on D2. This point is differ from the report of Tatsuya Tomo et al[10]. On protein D2, Car489 transfers energy to Chl *a*672, then to Chl *a*677 in 61 ps, the singlet state of Chl *a*677 decays to ground state in 3 ns and to triplet state in 10 ns. On protein D1, Car507 transfers energy to P680 via other pigments in 274 ps. The recombinations of $P680^+$ Pheo a^- occur in long time 3.8 ns and 16 ns.

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采用选择激发研究光系统Ⅱ反应中心 β-Car 的物理特性

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摘 要 光系统Ⅱ反应中心包含有2个去镁叶绿素分子(Pheo),2个β胡萝卜素分子(β-Car)和6个叶绿素a分子(Chla). 对反应中心的时间分辨荧光光谱表明,两个β-Car具有不同的吸收光谱,吸收峰分别为489nm (Car489)和507 nm(Car507),Car489 靠近吸收峰为667 nm和675 nm的叶绿素 a(Chl a),它的主要功能是保护反应中心免受单态氧的破坏,而不能将激发能传递给光化学反应活性的色素分子 P680;Car507 靠近吸收峰为669 nm的 Chl a 分子;能够将激发能传递给 P680,进行电荷分离. 采用全局优化拟合的方法对荧光光谱进行处理,Car489 在61 ps时间内将能量传递给 Chl a672, 随后传给 Chl a677,处于激发态的 Chl a677 在3 ns衰减到基态;Car507 在274 ps时间内将能量传递给 P680,$P680^+Pheo^-$的电荷重组发生在3.8 ns和16 ns.

关键词 光系统Ⅱ;反应中心;β-Car;电荷重组;能量传递

He Junfang was born in Shanxi, China on November 27, 1971. She graduated from physics department of Huaibei Coal Teachers College and got B. S. degree in 1993. She got M. S. degree and Ph. D. in Xi'an Institute of Optics and Precision Mechanics, Chinese Academy of Sciences in 1996 and 1999, respectively. She is mainly interested in ultrafast phenomenon between light and material, such as photobiological and photophysical. Now her interests are in photosynthesis and nanocrystals.