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Pigment stoichiometry of the Photosystem II reaction center from higher plants

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Abstract

A preparation of Photosystem II reaction center complex containing 4 chlorophylls per 2 pheophytins was obtained from three different species of higher plants. A 'standard' reaction center preparation (6 Chl/2 Pheo) was used as starting material. This material was loaded onto a sieve chromatography Superose-6 column run on a FPLC system. Three different sets of fractions were eluted from the column. The first few fractions showed a distinct absorption band at 435 nm and pigment content of 10 Chl per 2 Pheo. The peak fractions did not show a distinct band at 435 nm, but rather a shoulder, and contained 4 Chl per 2 Pheo. The few last fractions exhibited relatively higher absorption at 416 nm, most probably due to higher cytochrome content, but they also contained 4 Chl per 2 Pheo. Similar results were observed when a reaction center preparation of about 5 Chl was loaded onto the Superose-6 column. This indicates that the 'standard' Photosystem II reaction center preparation is heterogenous, composed of 4 Chl reaction centers and contaminants containing higher pigment content. To further validate this conclusion, we obtained a 4 Chl preparation by modification of the 'standard' reaction center purification procedure. After loading this preparation onto the Superose-6 column, one single chromatographic band was obtained, and all fractions contained 4 Chl per 2 Pheo. This strongly suggests that a 4 Chl per 2 Pheo preparation corresponds to that of a native core Photosystem II reaction center preparation.

Key words: Chlorophyll; Chromatography; FPLC; Pheophytin; Photosynthesis; Reaction center; Pigment stoichiometry

1. Introduction

Due to sequence similarities between the D1-D2 heterodimer of oxygenic photosynthetic organisms and the L-M subunits of purple photosynthetic bacteria [1,2], it was thought that D1-D2 constituted the core of the Photosystem II (PS II) reaction center (RC). The isolation of the PS II RC by Nanba and Satoh [3], and its stabilization with the detergent dodecyl maltoside [4] has facilitated many detailed biochemical and biophysical studies on this complex chromoprotein (for recent reviews see Refs. [5] and [6]). The isolated PS II RC complex actually consists of several polypeptides [3], including D1, D2, the α- and β-subunits of cytochrome (Cyt) *b*-559, and the *psbI* gene product [7]. As in the case of the purple bacterial L-M heterodimer [8], D1-D2 probably binds all the pigments (chlorophyll [Chl], pheophytin [Pheo], and carotenoids [Car]) within the PS II RC. This was experimentally demonstrated by Tang et al. [9].

The exact chromophore stoichiometry within the PS II RC, however, is still the subject of much debate. The question is relevant in the study of excited electronic states, primary charge separation, and energy transfer dynamics within the RC. The first preparations of isolated RC contained between 4–5 Chl, and 1 β-Car per 2 Pheo [3,10]. More recently, preparations with higher pigment content were reported, usually consisting of 6 Chl and 2 β-Car per 2 Pheo [11–14]. Montoya et al. [13,15] described the isolation of two preparations of PS II RC with different pigment content depending on the column washing time used. One

Abbreviations: Car, carotene; Chl, chlorophyll; Cyt, cytochrome; FPLC, fast performance liquid chromatography; MW, molecular weight; Pheo, pheophytin; PS, Photosystem; RC, reaction center.

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contained 6 Chl, and 2 β-Car per 2 Pheo, and the other contained 4 Chl, and 1 β-Car per 2 Pheo. At this time, we proposed that the extra pigments (2 Chl and 1 β-Car) corresponded to those located on the surface of the protein complex and thus were more easily removed by the buffer wash. More recently, other evidence indicated that the extra pigments could come from contamination of the RC preparations by other pigment-protein complexes and even by free Chl (Chang, Jankowiak, Reddy, Yocum, Picorel, Seibert and Small, submitted to J. Phys. Chem.).

In this study we have tried to reassess the question of pigment stoichiometry. To do this the PS II RC was isolated and purified from three different species (soybean, sugarbeet, and spinach) under various experimental conditions, and then subjected to sieve chromatography using a FPLC system. The results demonstrate that independent of the kind of preparation loaded onto the sieve chromatography column, RCs with 4 Chl per 2 Pheo were obtained. On the other hand, the first fractions off the column showed a much higher Chl content than latter fractions.

2. Materials and methods

Reaction center preparations were obtained from chamber-grown soybean and sugarbeet, and from market spinach. The RC complex was purified according to the procedure of Nanba and Satoh [3] as modified by Seibert et al. [4] and Montoya et al. [13], with the exception that 60 mg instead of 30 mg of Chl were loaded onto the ion-exchange column, and a Q-Sepharose Fast Flow column [16] was used instead of Fractogel TSK 650s. In the case of 'standard' RC preparations (6 Chl/2 Pheo), the column was washed until the peak of the eluate at 435 nm (mainly due to Chl) was a little lower than that at 416 nm (due to Chl, Pheo, and cytochrome *b*-559). To obtain RC preparations with lower pigment content, a longer washing time was needed, which further reduced the absorption at 435 nm compared to that at 416 nm (Ref. [13]; Chang, Jankowiak, Reddy, Yocum, Picorel, Seibert and Small, submitted).

After elution from the ion-exchange column, 'standard' RC preparations were further purified by sieve chromatography with a Superose-6 HR 10/30 column (resolution range $5 \cdot 10^3$–$5 \cdot 10^6$ MW) using a FPLC system (Pharmacia). The column was equilibrated with 50 mM Tris-HCl (pH 7.2) containing 0.6 mM dodecyl maltoside. Sample (200 μl of 30 $\mu g \cdot (ml\ Chl)^{-1}$) was loaded onto the column and eluted at a flow rate of 0.05 $ml \cdot min^{-1}$ with the same buffer. The eluate was collected in fractions of 50 μl. Only the pigmented fractions are represented in the elution profiles shown in Figs. 1 and 3. All procedures were done in the dark at 4°C. When necessary the preparations were concentrated using an Amicon Stirrer Cell or Centricon-30 concentrator tubes.

Pigment stoichiometry was assayed as in Montoya et al. [13] using a millimolar extinction coefficient for the Pheo of 49.3 and 6.504 at 665.5 nm and 535 nm, respectively.

3. Results and discussion

Following the 'standard' procedure, a RC preparation containing around 6 Chl per 2 Pheo was obtained. The spectrum of this preparation at room temperature was independent of the plant species (soybean, sugarbeet, or spinach) used as the starting material and was quite similar to that published earlier [11–14]. The characteristics of this 'standard' spectrum were the presence of a main red band peaking at 675.5 nm (due to the Q_y transitions of the Chl and Pheo), a small band at 543 nm (due to the Q_x transition of Pheo), a band at 435 nm (due to the Soret transition of the Chl), and a band at 416 nm (due to Chl vibronic and the Soret transition of Pheo and cytochrome *b*-559). β-Car is responsible for absorption in the 510–450 nm range, but it also contributes significantly to the absorption at 435 nm. This fact could lead to misleading conclusions on the Chl content of different RC preparations, if only the absorption intensity at 435 nm is compared. This is especially critical since it is known that β-Car can easily be washed out from the RC complex [13,15, 17].

When the 'standard' preparation (6 Chl/2 Pheo) was passed through a Superose-6 column, several pigmented fractions were eluted. Fig. 1 shows the elution profile measuring the absorption intensity at 675 nm.

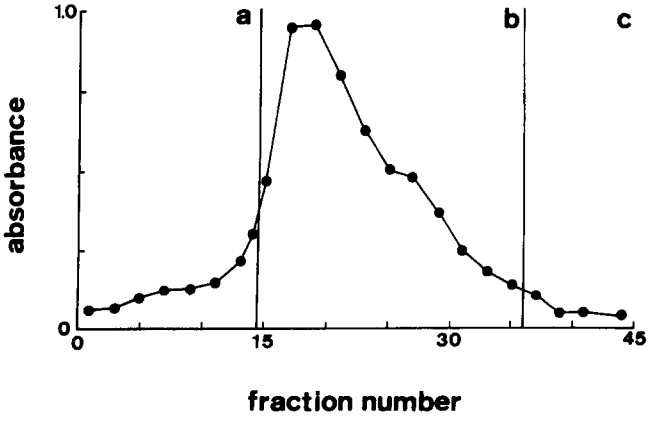

Fig. 1. Elution fraction profile of the absorption at 675 nm from the sieve chromatography Superose-6 column loaded with a 6-Chl Photosystem II reaction center preparation from soybean. Each fraction contained 50 μl of sample. Fractions a, b, and c correspond to the three sets of fractions described in the Results and discussion section.

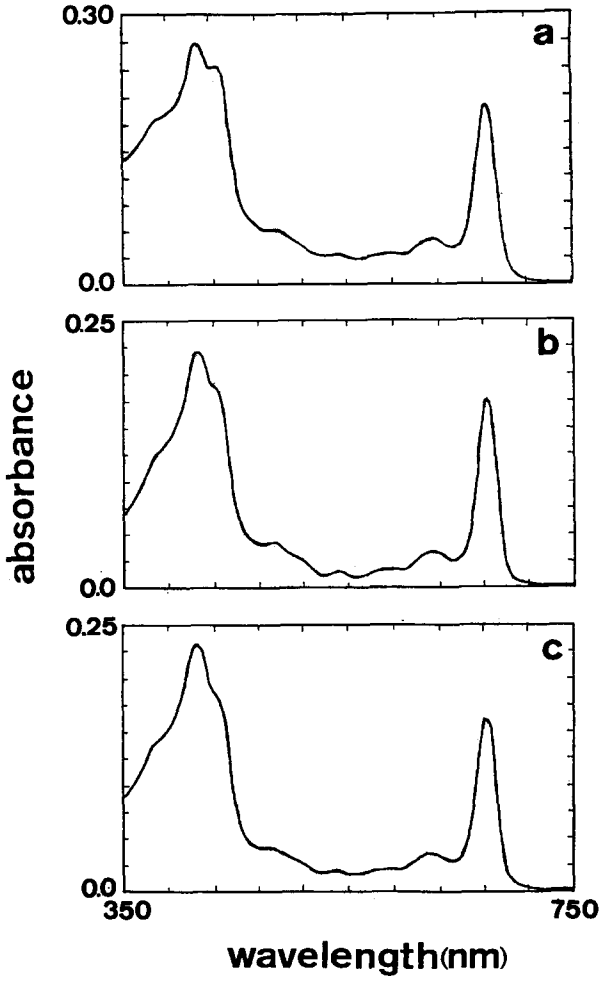

Fig. 2. Absorption spectra of the three different sets of fractions obtained from the Superose-6 column. (a) Resultant spectrum from the combined fractions of Fig. 1a; (b) Resultant spectrum from the combined fractions of Fig. 1b; (c) Resultant spectrum from the combined fractions of Fig. 1c. The preparations were resuspended in 50 mM Tris-HCl (pH 7.2) plus 2 mM dodecyl maltoside.

Three different kinds of sample were eluted. The absorption spectra of the first few fractions (a) were similar to that of the loaded sample but with a more distinct and pronounced 435 nm band. These fractions were pooled and concentrated, and the resultant spectrum with a maximum around 675 nm is shown in Fig. 2a. The peak fractions had similar spectra with the exception that the band at 435 nm was not distinct. It appeared as a shoulder. The fractions (b) with this kind of spectrum were also pooled and concentrated, and the resultant spectrum with maximum at 675 nm is shown in Fig. 2b. Note that the different absorption profile at 435 nm in Fig. 2b was not the result of a lower β-Car content since the absorption at around 485 nm due to this pigment was similar in both samples. The latter fractions of the chromatogram (c) exhibited a spectrum similar to that of Fig. 2b but with higher relative intensity at 416 nm. In addition, the red peak was slighly blue shifted (maximum at around 673 nm). All these fractions (c) were pooled and concentrated, and the resultant spectrum is shown in Fig. 2c. Although we did not further analyze the preparation resulting from fractions (c) we think that the higher intensity at 416 nm is most probably due to a higher cytochrome *b*-559 content and that the preparation is partially deactivated [4,5]. Similar spectra were also observed in the latter fractions where Fractogel TSK 650s chromatography was used in the 'standard' RC purification procedure (Toon, Picorel and Seibert, unpublished results), and when the short-Triton treatment of Dekker et al. [18] was used.

The pigment content for each set of samples corresponding to the spectra in Figs. 2a, 2b and 2c, was determined to be 9.9, 4.0 and 4.1 Chl per 2 Pheo, respectively. Furthermore, the addition of the total Chl content of the three set of samples was close to that loaded onto the Superose-6 column, which indicates that little pigment is lost in the sieve chromatography step. These results strongly suggest that the 'standard' preparation of PS II RC (6 Chl/2 Pheo) is heterogenous and can be separated into several sub-preparations with different pigment contents. More important, this also demonstrates that it is possible to obtain a preparation containing 4 Chl per 2 Pheo, which probably corresponds to that of the native core PS II RC. Ongoing experiments are attempting to identify biochemically the origin of the contamination of the 'standard' preparation of the reaction center (6 Chl/2 Pheo).

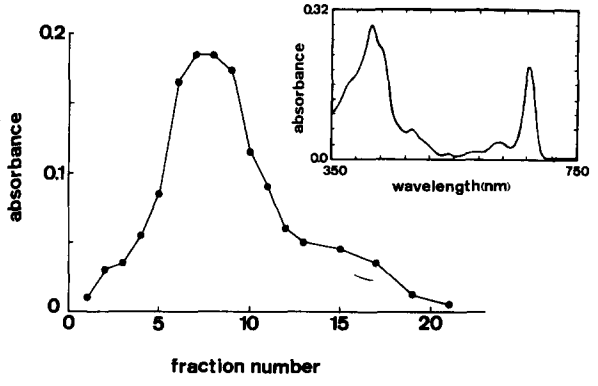

Fig. 3. Elution profile of the absorption at 675 nm from the sieve chromatographic Superose-6 column loaded with a 4-Chl reaction center preparation from sugarbeet. Each fraction contained 50 μl of sample. Note that a single band was obtained. Inset: absorption spectrum of the combined fractions from the chromatography; all of the fractions exhibited similar spectra.

To further validate the results presented above, we obtained a RC preparation with 4 Chl per 2 Pheo after washing the ion-exchange column for a long time, as described by Montoya et al. [13,15]. This preparation was loaded onto the Superose-6 column as indicated above. In contrast to what happens with the 6 Chl preparation, no fractions with high absorption at 435 nm were detected. All the fractions from this chromatography showed a stoichiometry equivalent to 4 Chl per 2 Pheo. This indicates that a preparation with 4 Chl per 2 Pheo corresponds to a homogeneous preparation of the PS II RC.

Preparations of RC with an intermediate pigment content can also be obtained by controlling the washing time during the ion-exchange chromatography step (Chang, Jankowiak, Yocum, Picorel, Seibert and Small, submitted). Following this procedure we were able to isolate a RC preparation with about 5 Chl per 2 Pheo. After loading this kind of sample onto the Superose-6 column, we were also able to obtain RC fractions containing 4 Chl per 2 Pheo, and some with higher Chl content (data not shown).

It should be pointed out that sieve chromatography did not resolve the fractions with different Chl content very well. Indeed, in the chromatogram profile shown in Fig. 1, it is obvious that one cannot see three well separated chromatographic bands. This indicates that the MW of the contaminant(s) plus the detergent micelle is higher but not very different from that of a 4 Chl RC plus the detergent micelle. To succeed with this type of chromatography, no more than 200 μl of sample should be loaded onto the Superose-6 column (1×30 cm), and the flow must be kept at a very low rate, about 0.05 ml min^{-1}.

All of the results described above indicate that a 6-Chl preparation is a heterogeneous preparation composed of RC complex with 4 Chl per 2 Pheo and other pigment-protein complexes with higher pigment content. We have also demonstrated that from a 6-Chl preparation it is possible to obtain fractions with different pigment stoichiometry. The pigment content of 4 Chl per 2 Pheo is equivalent to that found in the RC isolated from purple photosynthetic bacteria [8]. This strongly suggests that 4-Chl preparations correspond to that of the core, native RC of PS II.

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