Heterogeneity in photosystem Ⅰ from *Pisum sativum* L.

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Abstract Studies have demonstrated that there are two types of PSI (PSI-1 and PSI-2) particles in some higher plants. These two types of PSI particles are proved to differ in organization and distribution on thylakoid membranes. PSI-1 mainly exists in the non-partition regions of thylakoids, while PSI-2 mainly in the partition regions.

Keywords: ***Pisum sativum* L., photosystem Ⅰ (PSⅠ), chlorophyll protein complexes, polypeptide composition, heterogeneity in photosystem Ⅰ.**

IT has been widely acknowledged that there are two types of photosystem Ⅱ (PSⅡ) in chloroplasts[1,2].

However, the heterogeneity in photosystem Ⅰ has been in question for a long time. When Hill and Bendall proposed the concept of photosynthetic chain in the 1960s, it was once believed that the members of photosynthetic chain such as PS Ⅰ and PS Ⅱ were located in the same domain of thylakoid membranes. Sane *et al.*[3], based on the studies in the period, advanced a novel theory and pointed out that there were two types of PS Ⅰ in chloroplast: one participating in non-cycle photosynthetic phosphorylation was located in grana regions close to PS Ⅱ; and the other mainly participating in cycle photosynthetic phosphorylation was located in stroma lamellae. Although direct evidence was still absent for the existing of two types of PS Ⅰ in thylakoid, the idea of heterogeneity in PS Ⅰ has been put forward since then. Mullet *et al.*[4] at the beginning of the 1980s isolated a "native" PS Ⅰ complex for the first time. Initially, the particles were believed homogenous and distributed on the thylakoid membranes uniformly. Andersson *et al.*[5—7], set up their own opinion against Sane's, argued that PS Ⅰ is separated from PS Ⅱ and is mainly located in the stroma lamellae. PS Ⅱ particles are mainly located in the grana stacks, only few of which are closely adjacent to PS Ⅰ particles. The idea was strongly supported by later research of the distribution of PS Ⅰ and PS Ⅱ on thylakoid membranes using immunocytochemical technique[8]. By the end of the 1980s and at the beginning of the 1990s, Swedish scientists isolated thylakoid membrane fractions by sonication and aqueous polymer two-phase systems[1,9—12]. They proved that the PS Ⅰ and PS Ⅱ exist in both of the grana and stroma lamellae, but the PS Ⅰ particles in the two membrane domains are heterogeneous as well as the PS Ⅱ particles. The PS Ⅱ α particles solely exist in the core regions of the grana, while the PS Ⅰ α particles, which are larger than PS Ⅰ β particles, are only distributed on the grana end membranes and margins. PS Ⅰ β and PS Ⅱ β mainly exist in the stroma thylakoids. Their results obviously supported Sane's suggestion. However, because the selectivity of sonication fragmentation and the membrane fractions purity by two-phase systems are not promised, the probability that there are still PS Ⅰ particles in the appressed grana could not be excluded. The thylakoid membrane proteins of *Pinus tabulaeformis* carr. were solubilized in different concentration of digitonin solutions and separated by non-denatured electrophoresis in our studies. The results showed that low concentration of digitonin can only solubilize one kind of PS Ⅰ particles located in non-appressed thylakoid membranes, while high concentration of digitonin can solubilize two types of PS Ⅰ particles. Considering the result, it was concluded that there may be two types of PS Ⅰ particles in chloroplasts of *Pinus tabulaeformis* carr., one may exist in the grana partitions, the other may be in non-appressed membranes[1,2]. Further studies have demonstrated that these two types of PS Ⅰ, called PS Ⅰ-1 and PS Ⅰ-2 respectively, also exist in the thylakoids of *Populus tomentosa* carr., *Spinacia oleracia* L. and *Pisum sativum* L.[13].

The present observations were mainly based on the different solubilization characteristics of digitonin and Triton X-100 on thylakoid membranes to isolate PS Ⅰ particles, examine the polypeptides composition of PS Ⅰ complexes and know the heterogeneity in PS Ⅰ of *Pisum sativum* L. further.

1 Materials and methods

Two-week-old *Pisum sativum* L. seedlings grown in green-house.

Thylakoids and chlorophyll protein complexes were isolated, and the measurement of absorption spectrum, activity and the chlorophyll content of PS Ⅰ was conducted as described in ref. [13]. The solubilization of PS Ⅰ particles using Triton X-100 and the measurement of P700 content were done as described by Mullet *et al.*[4]. The methods of SDS-PAGE and 2D-IEF-SDS-PAGE described in ref. [15] were used to analyse the polypeptides composition of the PS Ⅰ particles. The Tricine-SDS-PAGE[14] was used for the separation of low molecular-weight polypeptides.

2 Results

(ⅰ) Isolation of PS Ⅰ particles. The preparation (C) of membrane protein of pea chloroplast was prepared using 100 mg digitonin/mg chl to solubilize the thylakoids combined with sonication. Preparation

1) He, P., Effect of light on photosynthesis characteristics of chloroplasts and needles of *Pinus tabulaeformis* carr., Ph. D. Thesis, Beijing: Beijing Forestry University, 1987.

2) Tong, N., Studies of photosystem Ⅰ of *Pinus tabulaeformis* carr, MS Degree Thesis, Beijing: Beijing Forestry University, 1989.

C was separated by deoxycholate(DOC)-(9%)PAGE, the results are shown in fig. 1(c)). There are 6 green bands and a yellow band on the gel. The thylakoids membranes were solubilized with 8 mg Triton X-100/mg chl. The mixture was stirred for 30 min at 20℃, and then centrifuged at 20 000 × g for 60 min. The chlorophyll protein complexes in the supernatant (preparation A) were separated by DOC-(9%)PAGE. There are 4 green bands on the gel (as shown in fig 1(a)). The R_f value of the first band was equal to that of the first band of preparation C. The sediment treated by Triton X-100 was washed with distilled water for several times until the washing solution become no green. Then the pellet was solubilized using 100 mg digitonin/mg chl, and the chlorophyll protein complexes in the solubilized solution (preparation B) were also isolated by electrophoresis. Only one green band was shown on the gel (see fig. 1(b)), its R_f value was equal to that of the second band of preparation C. The bands of A1, B1, C1—C3 on the gel show the special absorption spectrum of PS I complexes. According to Gao *et al*.[13], the complex in band A1, C1 belongs to PS I -1, in B1, C2 belongs to PS I -2. The PS I particles purified by electrophoresis show elevated activity, and their chl/P700 value descends (see table 1), which demonstrates that the DOC-PAGE can efficiently separate the PS I complexes, while Triton X-100 only solubilized the PS I -1. The function of Triton X-100 is just the same as that of the low-concentration of digitonin. The solubilization of PS I -2 particles perhaps needs special and stronger detergent.

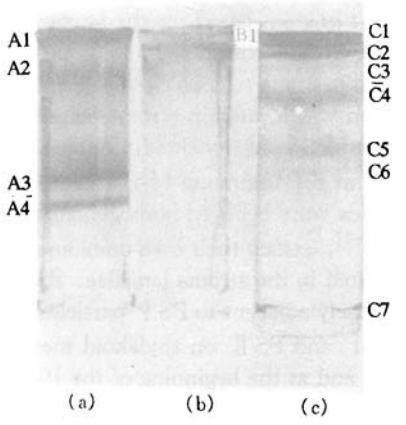

Fig. 1. Separation of membrane proteins by DOC-PAGE. (a) Membrane proteins of preparation A; (b) membrane proteins of preparation B; (c) membrane proteins of preparation C.

Table 1 Activity of PS I in preparations of pea thylakoid membranes

Preparations	Chl a/b	PS I activity/ μmol $O_2 \cdot mg^{-1}$ chl$\cdot h^{-1}$	Chl/P700
Thylakoid membranes	2.8	60	–
Preparation C	3.1	83	278
Preparation A	2.7	52	158
Preparation B	2.9	98	138
PS I -1 particles	3.3	214	45—48
PS I -2 particles	3.9	225	44—47

(ii) Separation of PS I polypeptides. The polypeptides of the two types of PS I complexes run out of the gel by electrophoresis were separated by 12% SDS-PAGE, as shown in fig. 2. The polypeptides of the PS I -1 and PS I -2 complexes are identical in the preparation C, the polypeptides of the PS I -1 complexes in the preparation C and A are similar. The 1st—4th bands on the separating gel of the preparation C were cut down and heat-denatured directly with SDS, then isolated by 16%—20% SDS-PAGE and Tricine-SDS-PAGE respectively. The results also show that the amount of PS I -2 polypeptides (fig. 3(a)-2 and (b)-2) is no less than PS I -1's (fig. 3(a)-1 and (b)-1), indicating that both PS I -1 and PS I -2 are relatively native PS I particles, while PS I -3 is a kind of PS I complex which depletes some polypeptides (fig. 3(a)-3 and (b)-3). The polypeptides of PS I -1 and PS I -2 complexes were separated by 2D-IEF-SDS-PAGE and stained with coomassie blue. The amount of the polypeptides of the two PS I particles was about 50 re-

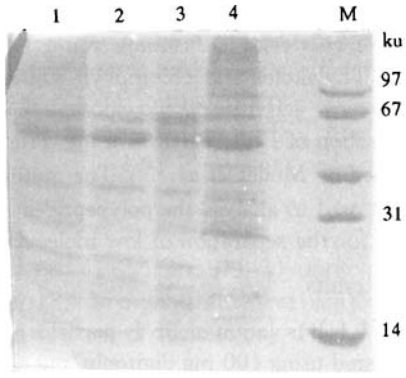

Fig. 2. Separation of PS I polypeptides by SDS-PAGE. 1, PS I -1 of preparation C; 2, PS I -2 of preparation C; 3, PS I -1 of preparation A; 4, thylakoid membrane proteins.

spectively, which displays no much difference, but some of them show definite difference in distribution and relative content on the gel as shown by arrows in figure 4.

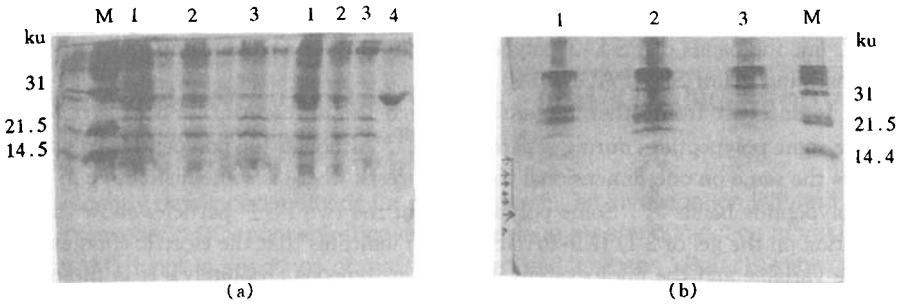

Fig. 3. Polypeptides composition of PS I particles. (a) Separated by 16%—20% SDS-PAGE; (b) separated by 16.5% Tricine-SDS-PAGE. 1, PS I -1; 2, PS I -2; 3, PS I -3; 4, LHCP1.

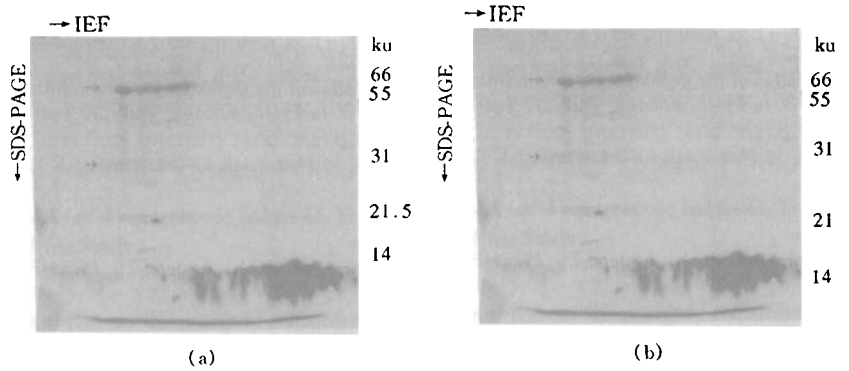

Fig. 4. Polypeptides of PS I -1 (a) and PS I -2 (b) separated by 2D-IEF-SDS-PAGE.

3 Discussion

Mullet *et al*.[4] obtained a native PS I complex (PS I -110) using the technique of minimal concentration of Triton X-100 solubilization and centrifugation of solubilized PS I on gradients, and this technique has been commonly used since then. We got two types of relative native PS I particles of *P. tabulaeformis* carr. by solubilization using high concentration of digitonin and separation by DOC-PAGE[1,2]. These two native PS I particles were also obtained from other higher plants, such as *Spinacia oleracia* L. and *Pisum sativum* L.[13]. A comparison of the effect of two detergents on PS I complexes solubilization proved that the Triton X-100 could only solubilize the PS I -1 particles, the PS I -2 particles could be dissolved from thylakoid membrane by treating the membrane remains with high concentration of digitonin. It is similar to the function of different concentrations of digitonin, the high concentration of digitonin could solubilize PS I -1 and PS I -2, low concentration of digitonin generally can solubilize PS I -1 particles which exist in non-appressed thylakoid membranes[13]. Our research proves that these two types of PS I particles differ in organization and distribution on thylakoid membranes, the PS I -1 particles mainly exist in the non-partition regions of thylakoids, while the PS I -2 particles mainly originate from the thylakoid partition regions. The suggestion is quite different from that of Swedish scientists, who recognized that all of the PS I complexes exist in the thylakoid non-appressed

1) See footnote 1) on page 59.
2) See footnote 2) on page 59.

regions[1,11,12].

In the present research, the obtainment of the two types of relative native PS I particles shows that the DOC-PAGE is an efficient method for isolating PS I particles compared with other ones. The size of PS I -2 particles is smaller than that of PS I -1 particles according to the isolation result by non-denatured electrophoresis, but the bands of PS I -2 polypeptides do not lose anyone compared with the bands of PS I -1 on the separating gel of SDS-PAGE.

Our result is different from other research in which the difference of PS I particle size is usually caused by losing some polypeptides during separating[4,16]. Although the polypeptide composition of PS I -1 and PS I -2 is the same on one-dimensional electrophoresis, there is some distinctive in the relative content of some polypeptide bands[13]. Some polypeptides of the two PS I particles show the definite difference in distribution on the gel of 2-D IEF-PAGE, which indicates that the electric charges carried on these polypeptides are variable and the photosystem I is heterogeneous absolutely. It is these electric charges that may affect the conformation and function of PS I particles.

The organization and structure of the PS I may be much more complex than that of having been recognized by the researches so far. The polypeptides organization of PS I particles may be variable in the different regions of thylakoid membranes, so the function of PS I particles will be diverse in various membrane regions.

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