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The light-independent biogenesis of photosystem in dark-grown lotus *Nelumbo nucifera Gaertn.* seedling

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Introduction

Lotus (*Nelumbo nucifera gaertn*) is one of the most primitive plants in the living angiosperms. Its embryo bud is enclosed by three layers of integuments (a hard exopleura, a thin endotesta and thick fleshy cotyledons). The embryo bud, which remains in the dark condition during its biogenesis process, can turn from pale yellow to bluish-green and synthesize and accumulate chlorophylls (Meeuse et al. 1962). Our previous investigation showed that even the chloroplasts in lotus embryo bud had giant grana, very low chl a/b ratio (0.7~0.9) and a major 30 KD polypeptide (Zuo et al. 1992), but they are still under-developed (Tang et al. 1999; Zuo et al. 1988). Studies on the germination and growth of lotus embryo bud in the light demonstrated that its chloroplast developmental pathway seemed to be different from that of other higher plants (Tang et al. 1999; 1993). Our present study is focused on the light-independent biogenesis of photosystem in dark-grown lotus seedling.

Materials and methods

Lotus (*Nelumbo nucifera gaertn*) seeds were collected from a farm in the western suburbs of Beijing. The seeds were germinated in water and kept in the dark for 10 days at 25 °C with water changed daily. Chloroplast thylakoids of the lotus embryo bud were prepared according to the method of Tang et al. (1993). The chlorophyll content was determined as in (Arnon, 1949).

Fluorescence emission spectra at 77K were recorded with Hitachi F4500 fluorescence spectrophotometer. Chlorophyll-protein complexes were analyzed by SDS-PAGE as in Ref. (Leto et al. 1980). The electron transfer rate measurement and Western Blots analyses were done according to the methods in Ref. (Tang et al. 1999; Luo et al. 1995) respectively. The antibodies of LHC were a kind gift from Prof. Stefan Jansson of Umeå University of Sweden.

Results

Changes of chlorophyll-protein complexes

SDS-PAGE analysis of chlorophyll-protein complexes of thylakoids prepared from lotus seed bud before germination was shown in Fig. 1. Only two green bands were resolved in "green gel". According to the absorbance spectra and the fluorescence emission spectra, they are monomeric LHCII and free pigment (FP) respectively. With western blots analysis (Fig. 2), it is showed that antibodies of Lhcb1 and Lhcb2 reacted with proteins from Spinach, lotus leaf and lotus seed bud, but antibodies of Lhca1 and Lhca4 didn't reacted with lotus seed bud. This indicated that LHCII protein was already existed in lotus embryo bud before its

germination, but none of Lhca1 and Lhca4. With the chloroplast from lotus seedling dark-grown for 10 days, four green bands could be resolved in green-gel (Fig.1). The upper one was named CPI, which included part of PSI

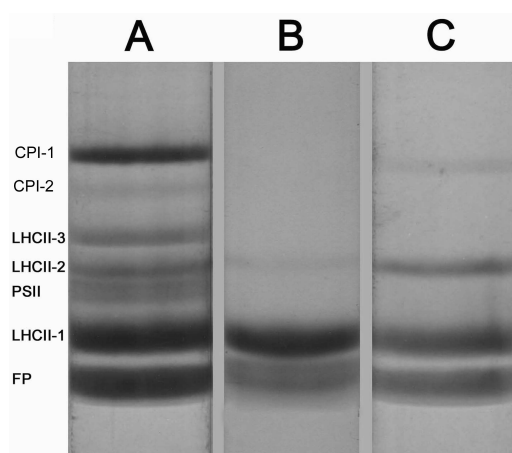


Fig.1 The partial denaturing SDS-PAGE of chloroplast. **A**, lotus leaf; **B**, lotus bud before germination; **C**, lotus seedling dark-grown for 10 days.

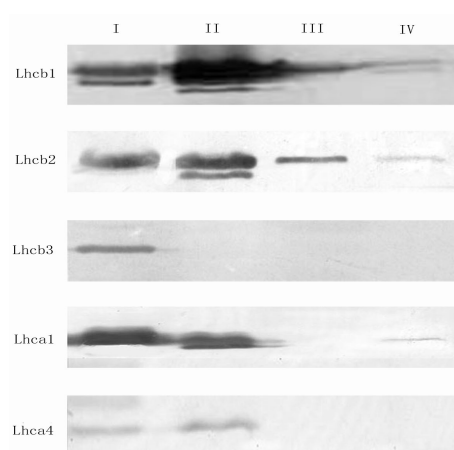


Fig.2 Western Blots analysis of LHC protein. **I**, spinach; **II**, lotus leaf; **III**, lotus bud before germination; **IV**, lotus seedling dark-grown for 10 days.

Changes of fluorescence emission spectra

Detection of fluorescence emission spectra at 77K showed that lotus embryo bud had only single emission peak at 678nm, no peak at region above 700 nm (Fig.3), which indicated that lotus embryo bud before germination had no PSI. The fluorescence

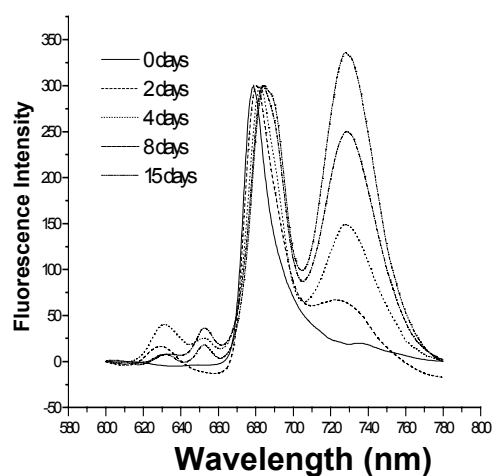


Fig.3 The fluorescence emission at 77K of lotus seedling during its dark-grown process.

emission peak at 725nm, emitted from PSI, begin to appeared on the 2th day after germination and increased gradually with prolong of the germination in the dark, at the same time, the peak at 678nm red shifted to 682nm while the peak at 725nm moved to 730nm (fig.3).

Western blots also demonstrated the existence of Lhca1 protein, which is part of the source of PSI fluorescence emission at 730nm (Fig.2).

Changes of chloroplast photosynthetic activities

The electron transfer activities were measured with H_2O , DCPIPH₂ as electron donor and with DCBQ, MV as ultimate electron acceptor of PSII and PSI, respectively. The results are summarized in Table 1 and 2.

Table 1 Electron transfer rate of lotus seed bud chloroplast during germination in the dark.

sample (days of germination)	electron transfer rate ($\mu\text{MO}_2/\text{mg chl h}$)	
	$H_2O \rightarrow \text{DCBQ}$	$\text{DCPIPH}_2 \rightarrow \text{M.V}$
2	0	0
10	0	211
lotus leaf (in the light)	61	256

Table 2 DCIP photoreduction activity of lotus seed bud chloroplast

sample (days of germination)	DCIP photoreduction ability ($\mu\text{MO}_2/\text{mg.chl.h}$)	
	-DPC	+DPC
10 days in the light	56.29	60.32
10 days in the dark	0	27

As show in Table 1, chloroplast from 2-day germinated lotus seed bud did not have photosynthetic activity. Electron transfer rate from H_2O to DCBQ and from DCPIPH₂ to MV couldn't be detected. Chloroplast isolated from dark-grown lotus seedling for 10 days had PSI reduce activity, which corresponds to 82% of the activity in fully developmental chloroplast of lotus leaves (Table 1), but no PSII oxygen evolution activity. After addition of electron donor DPC, the light-driven DCIP reduce activity could be detected (Table 2). Conversely, lotus seedling germinated and grown in the light for 10 days, which already formed PSII, had DCIP reduce activity in the absence of DPC (Table.2).

Discussion

It is well known that the formation of photosystem needs two conditions: chlorophyll and apoprotein. Angiosperms need light to form chlorophyll and apoprotein(Kreuz et al. 1986; Sutton et al.1987), so photosystem biogenesis is not found in dark-grown angiosperm seedlings. Lotus (*Nelumbo nucifera*), which has a unique position in the phylogeny of the angiosperm, is special in that its seeds contain chlorophylls(Meeuse et al.1962; Zuo et al.1988). These chlorophylls could be used to form photosystem in lotus seedling during germination in the dark. The key question is that whether proteins assembling with chlorophyll could be synthesized and accumulated in Lotus.

Many processes involved in the translation and expression of Reaction Center proteins and interior antenna proteins encoded by plastid genes are regulated by light. But there are other studies indicated that illumination is not necessary for their expression (Mullet et al.1990; Zichacker et al.1990). This can also be proven by the fact that lotus can germinate in the dark with photosystem establishment.

The activation of nuclear genes encoded exterior antenna proteins, LHCI and LHCII, is dependent on light in most angiosperms (Thompson et al. 1991). However, in some angiosperms, the transcription expression of LHC is not dependent on light, such as barley and *Arabidopsis* (Banmgartner et al. 1989; Brusslan et al. 1992). Lotus plumule can synthesize LHC in the dark, suggesting that the transcription expression of LHC is not dependent on light, either.

In conclusion, Lotus plumule has already had the condition of forming the photosystem in the dark. Our results also demonstrated completely that the Lotus plumule germinated and grown in the dark for 10 days have already formed photoactivated PSII and PSI, but the water-splitting system of PSII is underdeveloped.

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