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Properties of Photosystem II Particles Prepared from Chloroplasts of Spruce Seedlings

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Photosystem II particles were prepared from spruce chloroplasts, using low concentrations of Triton X-100. Properties of the particles such as low chlorophyll ***a/b*** ratio (2.2), slight activity of Photosystem I dependent O_2 uptake and decrease of fluorescence emission band at 732 nm indicate that Photosystem I components were selectively removed by the detergent treatment.

The Photosystem II particles obtained from the chloroplasts of seedlings grown in the dark were devoid of only O_2-evolving activity. However, the particles prepared from chloroplasts, which were isolated after preillumination with weak white light of the seedlings, showed high activity of O_2 evolution. These two preparations showed identical polypeptide patterns of SDS-polyacrylamide gel electrophoresis. The results suggest that the absence of O_2-evolving activity in the spruce grown in the dark is not due to the lack of polypeptides responsible for the hydrolysis of water.

INTRODUCTION

The greening of angiosperms is dependent on light. On the other hand, gymnosperms have the ability to develop their chloroplasts during germination in the dark. We have shown previously that the chloroplasts of spruce grown in the dark have unstacked thylakoid membranes, and that the electron transport systems of PS I and II are set up in the chloroplasts (Oku et al., 1975). Such chloroplasts are, however, devoid of O_2-evolving activity (Oku and Tomita, 1976 and 1980).

It was found that the O_2-evolving activity appears when the spruce seedlings grown in the dark were exposed to continuous light or repetitive flashing light (Oku and Tomita, 1976 ; Inoue ***et al.,*** 1976). Similar light-dependent activation was observed in broken chloroplasts isolated from spruce seedlings grown in the dark (Oku and Tomita, 1980). When broken chloroplasts were exposed to red light for 30 min in the presence of reductant ($DCIPH_2$) and divalent cation (Ca^{2+}), photoactivation of the latent water-splitting system occurred as effective as did in seedlings. The results suggest that the activation proceeds via simple

Abbreviations : Chl, chlorophyll ; PS I, Photosystem I ; PS II, Photosystem II ; MV, methylviologen ; PBQ, phenyl-*p*-benzoquinone; DCIP, 2, 6-dichlorophenyl indophenol ; DCMU, 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea ; SDS, sodium dodecyl sulfate.

events, which involve, at least, reduction of water-splitting enzyme complex. In order to clarify the photoactivation process, more simplified system, but not broken chloroplasts, seems to be necessary.

Recently, O_2-evolving PS II particles have been separated by detergent treatment of chloroplasts (Akerlund *et al.*, 1976 ; Kuwabara and Murata, 1979 ; Toyoshima and Fukutaka 1982 ; Yamamoto et *al., 1982* and 1983). The PS II particles contain less protein components than those of broken chloroplasts. The PS II particles were thus employed to examine the photoactivation process in the present study. In this paper we describe a simple method of preparing the particles from spruce chloroplasts, which are active or inactive in splitting water. Biochemical properties of the particles are also described.

MATERIALS AND METHODS

Spruce (*Picea dies)* seedlings were grown on vermiculite for three weeks at 25 °C in the dark. Cotyledons of the spruce grown in the dark were harvested and used for the isolation of "dark chloroplasts", which lack O_2-evolving activity. To prepare "light chloroplasts", the spruce seedlings grown in the dark were illuminated with white light (10 W/m²) from fluorescent lamp for 30 min.

Broken chloroplasts were isolated from the dark-grown or illuminated cotyledons using a medium containing 50 mM Hepes-NaOH (pH 7.5), 350 mM sorbitol, 10 mM NaCl, 1 mM $MgCl_2$, 1 mM $MnCl_2$ and 10 % (W/V) polyethylene glycol-4000 by the method of Oku and Tomita (1980). The chloroplasts were suspended in a medium containing 25 mM Hepes-NaOH (pH 7.5), 100 mM sucrose, 5 mM NaCl and 1 mM $MgCl_2$, and then 0.5 ml of 2 % Triton X-100 was added to 5 ml of the chloroplast suspension containing 1 mg chlorophyll. After the Triton X-100 treatment for 12 min with stirring in an ice bath, the medium was centrifuged at 10,000 x g for 5 min to remove membrane fragments. The supernatant was again centrifuged at 27,000 x g for 10 min. The pellet which is PS II enriched fraction was resuspended in the above medium. All the procedures were carried out under dim green light. Chlorophyll concentration was determined by the method of Arnon (1949).

0, evolution and PS I dependent 0, uptake were measured at 25°C with a Clark-type oxygen electrode under illumination with saturating red light (200 W/m²). The reaction medium for 0, evolution contained 0.2 mM phenyl-*p*-benzoquinone and 0.1 mM ferricyanide in 3 ml of the isolation medium. 0, uptake as measured as PS I activity was assayed in the presence of 0.1 mM methylviologen, 50 μM DCIP, 0.5 mM sodium ascorbate, 10 μM DCMU and 1 mM KCN. PS II activity was followed by the absorbance change of 50 μM DCIP at 581 nm under the illumination with red actinic light (400 W/m²) in the presence and absence of 0.5 mM diphenylcarbazide (DPC). Broken chloroplasts and PS II particles were added to the reaction medium at the concentration equivalent to 50 μg chlorophyll for the assay of 0, evolution or uptake and 20 μg chlorophyll for DCIP photoreduction.

Fluorescence emission spectra at 77 K were measured with a Hitachi 850 fluorescence spectrophotometer. Excitation wavelength was 435 nm and the

half-bandwidth of the emission monochrometer was 1 nm.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (1970). Samples were dissolved in a solution containing 62.5 mM Tris-HCl (pH 6.8), 2 % (W/V) SDS, 10 % (W/V) sucrose, 5 % (V/V) mercaptoethanol and 0.001 % (W/V) bromophenolblue and then incubated in boiling water for 1 min. Chlorophyll content in the solution of 0.1 ml was adjusted to 10 μg in every sample. An aliquot of the solution was subjected to electrophoresis on a slab gel of 7.5-15 % linear gradient of acrylamide concentration. Electrophoresis was run at 200 V for about 5 hrs at 4°C. Gel was stained with Coomassie brilliant blue R-250, destained, and scanned at 565 nm with a Toyo digital densitometer DMC-33C.

RESULTS AND DISCUSSION

Effect of detergents such as Triton X-100, digitonin and deoxycholate on the preparation of O_2-evolving PS II particles was examined. Triton X-100 was most effective among these detergents in dissolving the thylakoid membranes. For 0.2 mg chlorophyll/ml, the treatment with 0.2 % Triton X-100 for 12 min was most suitable for obtaining the PS II particles with high activity of O_2 evolution and the lowest ratio of chlorophyll a to *b*. About 25 % of total chlorophyll present in the sample chloroplasts remained in the PS II particles.

Table 1. Ratio of Chl ***a/b*** and photochemical activities in chloroplasts and PS II particles. The activities of PS I and II are expressed by the rate of O_2 uptake and DCIP photoreduction, respectively. DCIP photoreduction was measured both in the presence and absence of 0.5 mM diphenylcarbazide (DPC).

Sample	Chl ***a/b***	O_2 evolution	PS II activity		PS I activity
			-DPC	+ DPC	
			(μmoles/mg Chl·h)		
Dark chloroplasts	3.4	0	3.3	36.6	97.8
Dark PS II particles	2.2	0	3.1	33.2	6.9
Light chloroplasts	3.4	57.3	—	—	168.8
Light PS II particles	2.2	59.2	—	—	7.1

The ratio of chlorophyll a/b and the photochemical activities determined in chloroplasts and PS II particles are summarized in Table 1. "Light PS II particles" showed the activity of O_2 evolution as high as that observed in "light chloroplasts". Neither the "dark chloroplasts" nor "dark PS II particles" obtained from the "dark chloroplasts" showed any activity of O_2 evolution. The activity of PS I was low in both dark and light PS II particles but it was high in both types of chloroplasts. The ratio of chlorophyll ***a/b,*** which was 3.4 in chloroplasts, decreased to 2.2 in particles isolated from both the light and dark chloroplasts. The results show that the content of chlorophyll *b* is high in the PS II particles, and that the pigment composition did not change during light illumination for 30 min of dark-grown seedlings.

The similar results were also deduced from the data in Fig. 1, where the

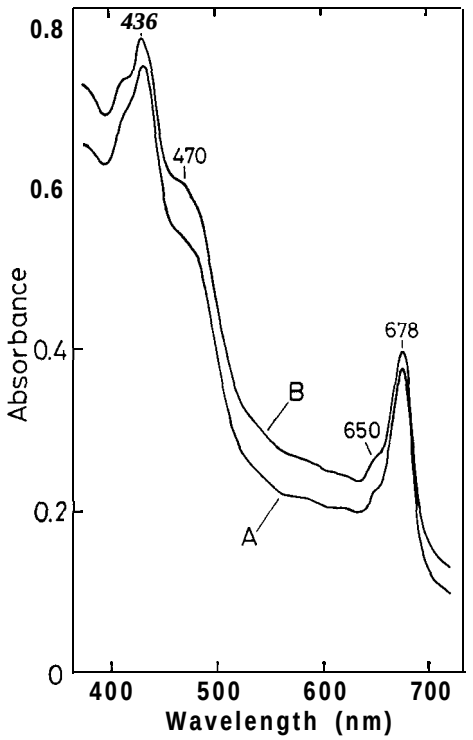

Fig. 1. Absorption spectra of "light chloroplasts" and "light PS II particles" measured at room temperature. A, chloroplasts ; B, PS II particles.

absorption spectra at room temperature of "light chloroplasts" and "light PS II particles" were compared. As shown by Curve B, shoulders of chlorophyll *b* absorption at 470 and 650 nm were prominent in the absorption spectrum of the PS II particles. But there was no significant difference between the absorption spectra of the PS II particles prepared from "light" and "darks" chloroplasts (data not shown). The results suggest that the pigment composition of PS II did not alter during illumination with weak light for 30 min of spruce seedlings grown in the dark.

Fig. 2 shows the fluorescence emission spectra of "light chloroplasts" and "light PS II particles" observed at liquid nitrogen temperature. The fluorescence intensity at 732 nm of PS II particles decreased strikingly, and the ratio of F732 (intensity at 732 nm) to F685 (intensity at 685 nm) was 0.56 in the chloroplasts and 0.23 in the PS II particles. The decrease of the 732 nm band in the PS II particles indicates that considerable amount of the PS I antenna chlorophyll was removed by the detergent treatment, because the fluorescence band at longer wavelength is emitted from the antenna chlorophyll in PS I (Butler and Kitajima, 1975).

In the chloroplasts of spruce seedlings grown in the dark, the electron

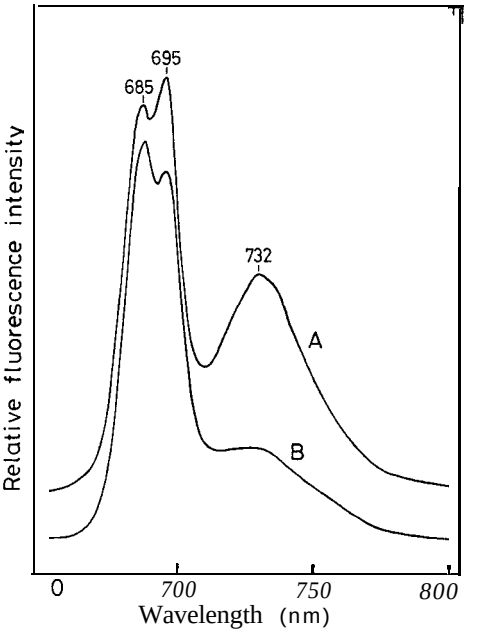

Fig. 2. Fluorescence emission spectra of "light chloroplasts" and "light PS II particles" at liquid nitrogen temperature. A, chloroplasts ; B, PS II particles.

transport systems of PS I and II were present, but the water-splitting system remained still inactive (Table 1). We showed previously (Oku et al., 1983) that Ca^{2+} added to the "dark chloroplasts" in the presence of reductant ($DCIPH_2$) is incorporated into thylakoid membranes during illumination, and that the incor-

Table 2. O_2-evolving activity after the photoactivation treatment of "dark chloroplasts" and "dark PS II particles". Samples in the medium containing 25 mM Hepes-NaOH (pH 7.5), 100 mM sucrose, 5 mM NaCl, 1 mM $MgCl_2$, 10 mM Na-ascorbate and 25 μM DCIP were incubated under illumination with white light (10 W/m^2) or in darkness for 30 min in the presence or absence of 5 mM $CaCl_2$. After the incubation, the samples were centrifuged at 27,000 x g for 10 min and resuspended in the reaction medium for the assay of O_2 evolution.

Sample	Conditions for photoactivation	O_2 evolution (μmoles O_2/mg Chl·h)
Dark chloroplasts		
	Dark (+5 mM Ca^{2+})	0
	Light	22.9
	Light ($5 mM Ca^{2+})	63.1
Dark PS II particles		
	Dark (+5 mM Ca^{2+})	0
	Light	0
	Light ($5 mM Ca^{2+})	0

poration of Ca^{2+} results in the photoactivation of latent water-splitting system. The effect of Ca^{2+} on photoactivation was examined in "dark PS II particles" and "dark chloroplasts".

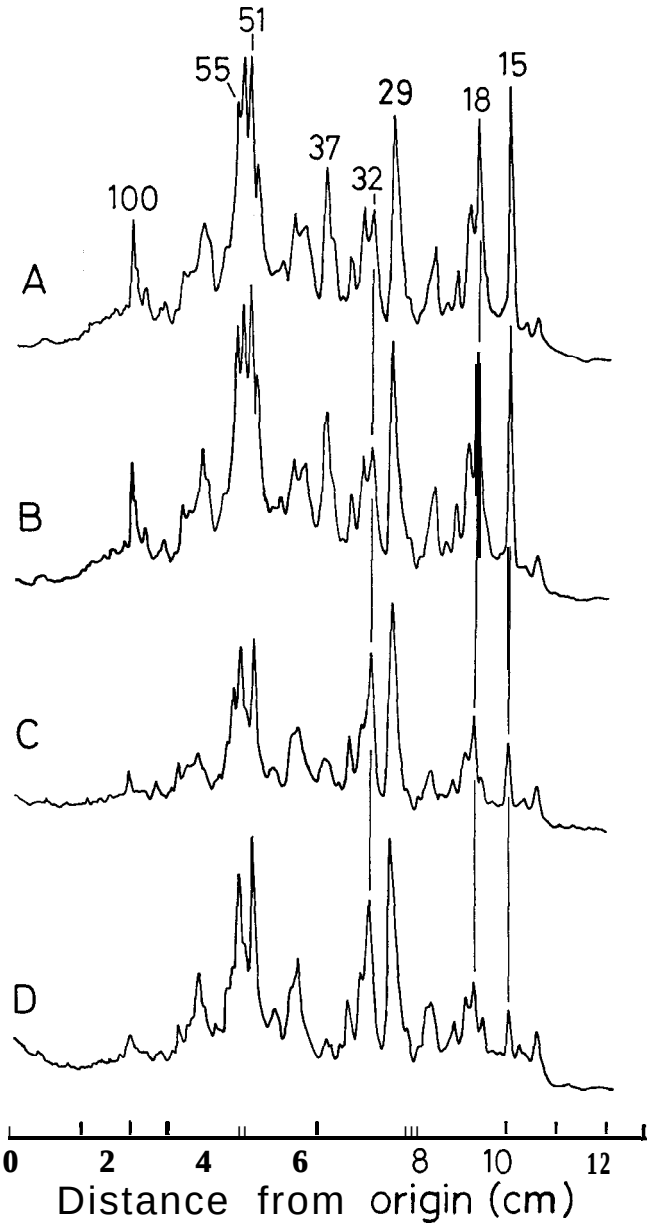

Fig. 3. Densitograms of polypeptides of chloroplast membranes and PS II particles separated by SDS-polyacrylamide gel electrophoresis. A, dark chloroplasts ; B, light chloroplasts ; C, dark PS II particles ; D, light PS II particles. The figures indicate the molecular mass of polypeptides in kDa.

As shown in Table 2, the O_2-evolving system of "dark chloroplasts" was activated by light illumination appreciably even in the absence of Ca^{2+}, but the activation was enhanced 3-fold by the addition of 5 mM Ca^{2+}. Such activation was not observed at all in the "dark PS II particles", when the particles were incubated under the illumination with white light for 30 min in the presence of 25 μM DCIPH, and 5 mM Ca^{2+}. There could be two possible explanations for this lack of photoactivation of PS II particles. One is that a factor in water-splitting enzyme complex, which is needed in the process of photoactivation, was lost during the detergent treatment of dark chloroplasts. Therfore, we examined the polypeptide compositions of the PS II particles and chloroplasts. Fig. 3 shows the densitograms obtained by SDS-PAGE of chloroplasts and PS II particles. As shown by Curves A and B, the polypeptide compositions of "light chloroplasts", were similar to those in "dark chloroplasts", and the components being responsible to 0, evolution, e. g., 15, 18 and 32 kD, were detected in both samples. Curves C and D show the densitograms for "dark PS II particles" and "light PS II particles", respectively. The polypeptide compositions of the dark PS II particles were identical with those of light PS II particles as far as judged from the patterns. These results suggest that all the protein components in water-splitting enzyme complex are present in the dark chloroplasts and the dark PS II particles.

The other possibility is that the acidification of intrathylakoid space or the formation of transmembrane electrochemical potential is indispensable for the photoactivation, because the photoactivation in Tris-treated chloroplasts is inhibited by the addition of uncoupler (Yamashita, 1982). We found that the photoactivation of "dark chloroplasts" depends on pH with the optimum at pH 7-8, which agrees with the optimum pH for photophosphorylation. As shown in Table 2, the photoactivation of water-splitting enzyme complex in water-splitting system of the "dark PS II particles" was not photoactivated by the activation treatment in spite of the fact that the treatment was attempted at the optimal conditions required by the "dark chloroplasts" ; namely, irradiation at the intensity of 10 W/m², incubation for 30 min at 25 °C, presence of adequate reductants (10 mM sodium ascorbate and 25 μM DCIPH,), addition of 5 mM $CaCl_2$ and pH at 7.5. The failure of photoactivation in the "dark PS II particles" may be attributed to the lack of membrane system or proton-accumulating system, which has probably an influence on the latent water-splitting system. Considering this possibility, we are planning to study the photoactivation of the latent water-splitting enzyme complex in the "dark PS II particles".

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