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ENRICHMENT OF PHOTOSYSTEM I REACTION CENTER CHLOROPHYLL FROM SPINACH CHLOROPLASTS

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Summary

The reaction center chlorophyll of Photosystem I in spinach chloroplasts was highly enriched. Preparations having 5—9 chlorophylls per 1 P700 were obtained by treating the Photosystem I particles prepared by digitonin treatment of chloroplasts with wet diethyl ether. All P700 present in the extracted particles was found to be photoactive, undergoing oxidation upon illumination.

In chloroplasts, the ratio of total chlorophyll to the reaction center chlorophyll, P700, is approximately 400 [1-3]. Kok first demonstrated that the reaction center chlorophyll of Photosystem I could be partially purified, to a ratio of chlorophyll to P700 of about 70, by selectively extracting light-harvesting chlorophyll from chloroplasts with a critical concentration of acetone [1]. Treatment of chloroplasts with detergents yielded subchloroplast particles which were enriched two- to four-fold in P700 [2, 3]. Ogawa et al. [4] showed that the application of Triton X-100 to photosynthetic membranes which were devoid of carotenoids produced small particles, HP700, with a chlorophyll to P700 ratio of 30. Photosystem I particles containing one P700 per about 100 chlorophylls were also prepared by mechanical disruption of chloroplasts with a French Press [5]. Sane and Park [6] obtained a further enrichment of the Photosystem I reaction center by treating the French-Press particles with acetone, obtaining a chlorophyll to P700 ratio of 16. This value, although up to now the lowest reported, indicates that the preparations still contain a significant amount of light-harvesting chlorophyll.

In this communication, we describe a procedure with which the reaction center of Photosystem I from spinach chloroplasts can be highly purified. Preparations having a chlorophyll to P700 ratio of 5—9 can be readily obtained by this procedure with a reasonably good yield. Some properties of the enriched preparation are also described.

Photosystem I particles which had been prepared by digitonin-treatment of spinach chloroplasts according to the method of Anderson and Boardman [7] were used as the starting material. The particles were twice washed with deionized water and then lyophilized. To 10 mg of lyophilized particles, 20 ml of chilled diethyl ether (75% saturation with water) were added and, after vigorous mixing, the precipitate formed was quickly separated by centrifugation. Ether extraction was usually repeated once. The extracted particles, which were almost white in appearance, were freed from ether by drying. The dried sample could be stored at 20°C for several months without any detectable change.

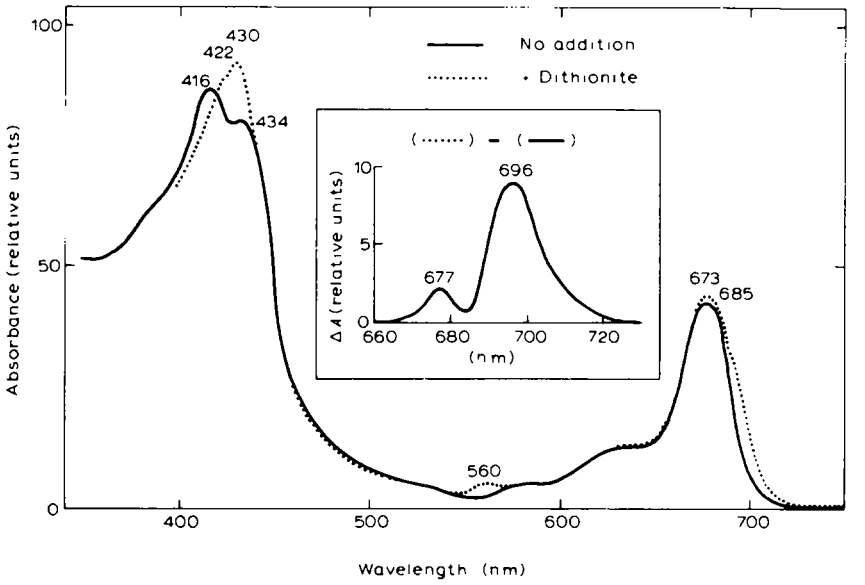

Fig. 1. Absorption spectra of the ether-extracted particles. Photosystem I particles were twice extracted with ether containing water at 75 % saturation. The chlorophyll to P700 ratio was 6. The spectra were determined with a Hitachi 356 Dual Wavelength Spectrophotometer in a split-beam mode. Since the sample scattered light, an end-on photomultiplier R375 was placed closely behind the cuvettes. In addition, "absorbance" at 750 nm, which was solely due to light scattering of the sample, was mechanically subtracted from the spectra. ——; no addition. - - - - -; a few grains of dithionite were added. Insert shows the difference between the two spectra.

Fig. 1 shows the absorption spectra of the ether-extracted particles. It is seen that the extracted particles still contain a small but significant amount of chlorophyll *a*. The red band of chlorophyll *a* shows an absorption maximum at 673 nm and a slight shoulder at 685 nm. The blue band shows a double peak, with absorption maxima at 416 and 434 nm. Absence of any appreciable peak or shoulder between 450 and 500 nm indicates that the particles are almost completely free from carotenoids and chlorophyll *b*.

The addition of a reducing reagent such as dithionite or ascorbate induced a marked change in the spectrum (dotted line in Fig. 1). There was a significant increase in absorbance at the longer wavelength region of the red band of chlorophyll *a*. A difference spectrum for the dithionite-induced absorbance

change (the insert of Fig. 1) shows two peaks with maxima at 696 and 677 nm, which is characteristic of P700 [3, 8]. The positions of the maxima are in good agreement with those reported with P700 in chloroplasts which had been treated with organic solvent or detergent [1, 3, 6]. The addition of ferricyanide did not change the absorption spectrum of the ether-extracted particles. This indicates that the particles contain P700 in the oxidized state.

The addition of dithionite also caused an absorbance increase at 560 nm, indicating the reduction of a *b*-type cytochrome. Absorption changes observed at the Soret region, i.e. an absorbance increase at 430 nm accompanied by a disappearance of the 416 nm peak, are ascribed to the reduction of both P700 and cytochrome *b*.

The original Photosystem I particles contained one P700 per 130 chlorophylls. Most of the chlorophyll was readily removed by the ether extraction. It was found, however, that the effectiveness of the ether extraction varied

TABLE I

CHLOROPHYLL AND P700 CONTENTS OF PHOTOSYSTEM I PARTICLES EXTRACTED WITH DIETHYL ETHER CONTAINING VARYING AMOUNTS OF WATER

Lyophilized Photosystem I particles were twice extracted with ether containing the indicated amounts of water. The water content in ether was varied by mixing water-saturated ether and ether which had been dried over anhydrous sodium sulfate. P700 content was determined from ascorbate-reduced minus ferricyanide-oxidized difference spectra. The difference molar extinction coefficient of P700 determined by Hiyama and Ke was employed [8].

	H_2O content in ether (% saturation)	Chlorophyll content (%)	Chlorophyll *a*/*b* ratio	P700 content (%)	Chlorophyll/P700 ratio
Photosystem I particles	—	100	7.0	100	130
Ether-extracted particles	0	20.0	4.6	50	50
	30	20.0	4.6	70	37
	50	12.0	5.0	70	20
	75	3.2	> 10	50	8
	100	2.6	> 10	35	9

markedly with the water content of the ether (Table I). After two extractions with dry ether, 20% of the chlorophyll remained. More chlorophyll was extracted, however, with wet ether. The maximum extent of pigment extraction was attained with ether which had been 75–100% saturated with water. The water content in ether also affected the chlorophyll *a* to *b* ratio of the extracted particles. Of special interest is a finding that P700 was highly resistant to the ether extraction, irrespective of the water content of the ether. About one half of the P700 originally present in the particles survived the extraction with wet ether, which removed 97% of chlorophyll. Thus, the extracted particles contained only 8–9 chlorophylls to one P700. The lowest value for the chlorophyll to P700 ratio so far obtained was 5. Our preparation is, therefore, two times more enriched in Photosystem I reaction center chlorophyll as the preparation of Sane and Park [6]. Repeated extraction with ether further decreased the chlorophyll content of the particles, but without further improving the chlorophyll to P700 ratio.

P700 in the ether-extracted particles was found to be photoactive. Fig. 2 shows the time course of light-induced absorbance change at 696 nm.

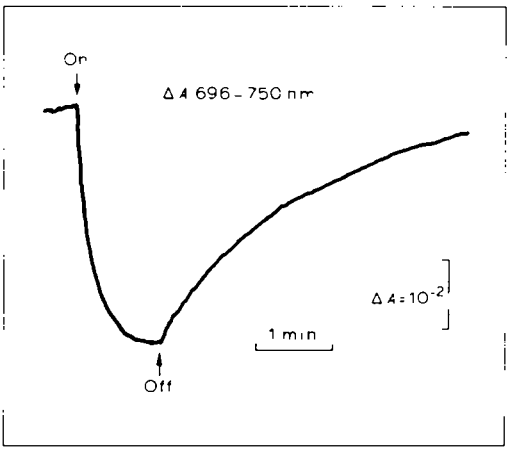

Fig. 2. Time course of the light-induced absorbance change at 696 nm in the ether-extracted particles. Absorbance changes were determined with the same spectrophotometer as used in Fig. 1, but in the dual wavelength mode. Reference wavelength was 750 nm. The sample was illuminated with blue light from a 650 W Halogen lamp through Corning 4-96, Toshiba VV-44 and Hoya HA-50 filters and a water layer of 5-cm thickness. The light-intensity was 2×10^5 ergs/cm^2 per s. To exclude scattered excitation-light, red filters (Toshiba VR-65 and VR-67) were inserted in front of the photomultiplier.

Ascorbate was added to the particles to reduce P700 prior to illumination. On illumination, the absorbance at 696 nm decreased and, after the light was turned off, it returned to the original dark level. The light minus dark difference spectrum for the light-induced change was similar in shape as well as in magnitude to the difference spectrum for the chemically induced absorbance change presented in Fig. 1. It is concluded therefore that all the reaction centers of photosystem I in the ether-extracted particles are in a photoactive state. The relatively slow rate of P700 photooxidation (see Fig. 2) might be due to the lower light absorption by the extracted particles which had lost most of the light-harvesting pigments.

The results presented in this report clearly indicate that extraction with wet diethyl ether is a simple method for the enrichment of the reaction center chlorophyll of Photosystem I. As compared with acetone, which has been used for the same purpose [1, 6], ether is much more selective in extracting the light-harvesting chlorophyll. With acetone-extracted spinach chloroplasts, a chlorophyll to P700 ratio of 70 was reported [1]. Ether extraction of the lyophilized spinach chloroplasts gave a chlorophyll to P700 ratio of 15. Another advantage of the present method is the reasonably good yield of P700; approximately one half of the P700 could be recovered in the ether-extracted preparations.

However, the present method does not allow any purification of the Photosystem I reaction center with respect to its protein constituents. Furthermore, the chlorophyll to P700 ratios of 5–9 imply that the preparation still contains some light-harvesting chlorophyll. Further purification is needed for the study of the chemical nature of the reaction center. At the present stage of purification, however, the preparation obtained in the present work would be, in virtue of its low pigment content, useful for the spectrophotometric investigation of the chemistry and photochemistry of the Photosystem I reaction center.

Acknowledgement

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