Biochimica et Biophysica Acta, 722 (1983) 492–497
Elsevier Biomedical Press

BBA 41264

RECONSTITUTION OF ANTENNA IN P-700-ENRICHED PARTICLES FROM SPINACH CHLOROPLASTS

ISAMU IKEGAMI

Laboratory of Chemistry, Faculty of Pharmaceutical Sciences, Teikyo University, Sagamiko, Kanagawa 199-01 (Japan)

(Received September 30th, 1982)

Key words: Photosystem I; P-700; Chlorophyll; Carotenoid; (Spinach chloroplast)

Reincorporation of photosynthetic pigments into chlorophyll-depleted and P-700-enriched particles prepared by ether extraction of lyophilized Photosystem I particles (Ikegami, I. and Katoh, S. (1975) Biochim. Biophys. Acta 376, 588–592) was carried out. On addition of the ether extracts to the P-700-enriched particles, 20–30 molecules of chlorophyll per P-700 were apparently incorporated into the particles in a form capable of transferring absorbed energy to P-700 with high efficiency. Carotenoids were also incorporated and can serve as antenna. The particles reconstituted with purified chlorophyll *a* showed a highly efficient energy transfer only when lipids, such as monogalactosyldiacylglycerol or phosphatidylcholine, were added together. Reconstitution with ether extracts also restored a long-wavelength-absorbing chlorophyll form with a maximum at about 700 nm and a prominent fluorescence emission band at a long wavelength at liquid nitrogen temperature.

Introduction

Chlorophyll is present in association with specific proteins in photosynthetic membranes in such a way as to be readily extracted with various organic solvents. Removal of chlorophyll from the thylakoid membranes is generally accompanied by an irreversible loss of photochemical activities due to the disorganization of the functional structure of the membranes. Kok [1] first demonstrated that part of the light-harvesting chlorophyll could be extracted with acetone without a significant loss of P-700 photooxidation. Sane and Park [2] succeeded in further lowering the chlorophyll content in the French press particles to give a preparation with a Chl/P-700 ratio of 16. More recently, Hirayama et al. [3] showed that extraction of PS I particles with a hexane/acetone mixture removed 78% of the chlorophyll and that reconstitution with Chl *a* led to a complete restoration of the activity of electron transfer from artificial electron donor(s) to methyl viologen.

Ikegami and Katoh [4] found that 95–98% of the light-harvesting chlorophylls could be extracted from PS I particles prepared from digitonin-solubilized thylakoids with wet diethyl ether, while keeping most of P-700 in the photoactive state. Thus, P-700-enriched particles with Chl/P-700 ratios as low as 6–9 were obtained. On continuous illumination, oxidation of P-700 proceeded slowly suggesting antenna of diminished size as a result of solvent extraction.

In the present work, we have examined whether the antenna can be reconstructed by adding photosynthetic pigments back to the P-700-enriched

Correspondence should be sent to the following address: Dr. Isamu Ikegami, c/o Dr. Bacon Ke, C.F. Kettering Research Laboratory, 150 East South College Street, Yellow Springs, OH 45387, U.S.A.

Abbreviations: Chl, chlorophyll; PS, photosystem.

particles. The results indicate that limited amounts of chlorophyll and carotenoids are reincorporated into the particles and consequently can serve as effective light-harvesting pigments of PS I. A far-red fluorescence band, which is characteristic of the antenna of PS I, was also restored and accompanied by the appearance of an absorption band near 700 nm in the reconstituted particles.

Materials and Methods

Preparation of P-700-enriched particles from spinach chloroplasts was described previously [4,5]. Briefly, PS I particles were first prepared by digitonin treatment of chloroplasts. The particles were lyophilized and then extracted twice with diethyl ether, 75% saturated with water, to yield P-700-enriched particles. The ether extracts contained about 95 and 70% of chlorophyll and lipids, respectively, present in the original PS I particles.

Pigments were reincorporated into the P-700-enriched particles by the following two methods. (1) The ether extracts (approx. 50 ml) obtained with 10–20 mg lyophilized PS I particles were dehydrated with anhydrous sodium sulfate, evaporated to dryness and dissolved in less than 0.5 ml ethanol. The ethanol solution which contained about 2–5 mg Chl *a*/ml was added dropwise (volume of each drop about 0.05 ml) to the P-700-enriched particles suspended in 20 ml phosphate buffer (10 mM, pH 8.0) with stirring. The initial concentration of Chl *a* in the suspension was 0.2–0.5 μg/ml. After incubation for 10–15 min at 0–4°C, the suspension was centrifuged at $24000 \times g$ for 15 min to separate the particles from free pigments remaining in the supernatant. The particles were suspended in the same phosphate buffer and used for subsequent photochemical analysis. In some cases, where mentioned, the pigment-incorporation process was carried out twice (see Table I). Chl *a*, which was purified from ether extracts by a column of powdered sugar and with petroleum ether containing *n*-propanol (0.5%, v/v) as elution solvent [6], was added to P-700-enriched particles as described above. (2) The second method consists of mixing lyophilized P-700-enriched particles with the ether extracts (obtained from digitonin-fractionated PS I particles). Before mixing, the ether extracts were dehydrated and concentrated to about 5 ml. The ether was then dried by evaporation below 4°C. The dried powder was washed once with 10 mM phosphate buffer (pH 8.0). The two methods yielded similar reconstituted particles, except that the amount of the pigments reincorporated was slightly greater by the second than by the first method.

Monogalactosyldiacylglycerol was prepared from spinach leaves by the method of Allen and Good [7]. Phosphatidylcholine and phosphatidylethanolamine were purchased from E. Merk AG, and Sigma Chemical Co., respectively.

Fluorescence and absorption spectra and P-700 photooxidation were determined as described previously [5].

Chlorophyll was estimated by the method of Arnon [8].

Results and Discussion

P-700 photooxidation

Table I compares chlorophyll contents and P-700 photooxidation before and after the ether extraction of PS I particles. The initial rate of P-700 photooxidation in the P-700-enriched particles was 8.7% of that in the PS I particles under continuous illumination with 430 nm light. Note that ether extraction caused a comparable decrease in the chlorophyll content; chlorophyll remaining in the P-700-enriched particles was 5.5% of that originally present in the digitonin particles. Thus, a lowered rate of P-700 photooxidation is apparently related to the decrease in the ligh-harvesting capacity. As an approximate measure of the relative quantum efficiency of the P-700 photooxidation, the initial photooxidation rates are normalized to the 430 nm absorbance of the particles. The value determined in P-700-enriched particles was 87% of that in PS I particles. This indicates that the antenna chlorophyll remaining in P-700-enriched particles could transfer the absorbed light energy to the reaction center of PS I with an efficiency comparable to that in the original PS I particles and that the rate of P-700 photooxidation determined by continuous illumination with blue light is approximately proportional to the size of the antenna.

The P-700-enriched particles used in this work had a Chl/P-700 ratio of 12. When the particles

TABLE I

PHOTOCHEMICAL PROPERTIES OF PS I PARTICLES, P-700-ENRICHED PARTICLES AND RECONSTITUTED PARTICLES

The P-700 contents were determined from the light-induced absorbance changes at 697 nm in the presence of 5 mM ascorbate, using the difference molar extinction coefficient of P-700 obtained by Hiyama and Ke [21]. The blue excitation light of 430 nm (half-band width, 14 nm) was supplied from a 650 W halogen lamp equipped with an infrared-absorbing filter (Hoya HA-50), two blue filters (Hoya V-440 and Corning 4-96), an interference filter (Nihon Shinku Kogaku Co, $\lambda_{max} = 430$ nm) and a 5 cm thick water layer. Light intensities used were between 100 and 1000 erg·cm^{-2}·s^{-1} where the oxidation rates were proportional to light intensity. The initial rates were normalized with the excitation light intensities. As an approximate measure for the relative quantum efficiency of P-700, the initial rate was divided by the absorbance at 430 nm. MGDG, monogalactosyldiacylglycerol; PC. phosphatidylcholine; PE, phosphatidylethanolamine; lipid/Chl ratios were 10 (w/w).

Fractions	Chl (%)	Chl/P-700 (mol/mol)	Initial rate of P-700 photooxidation (%)	Relative quantum yield of P-700 photooxidation (%)	Absorbance maximum (nm)
PS I particles	100	120	100	100	678
P-700-enriched particles	5.5	12	8.7	87	674
Reconstituted particles with					
ether extracts	13.4	29	20	83	672
ether extracts, twice [a]	35.3	88	27	37	669
Chl *a*	13.3	29	14	60	672
Chl *a*, twice [a]	38	83	18	26	670
Chl *a* + MGDG	13.4	29	22	91	672
Chl *a* + PC	11.0	25	19	91	672
Chl *a* + PE	5.5	12	8.4	84	674

[a] Reconstitution was repeated once more.

were reconstituted with ether-extracted pigments, the ratio increased to 29. The relative quantum efficiency of P-700 photooxidation was not significantly lowered in the reconstituted particles, because the initial rate of P-700 photooxidation was also increased by more than 2-fold. Clearly, reincorporated chlorophyll can transfer its excitation energy to P-700 with a high efficiency. The recombination of pigments also significantly stabilized the activity of P-700 photooxidation in the particles.

When the pigment-incorporation process was repeated once more, the Chl/P-700 ratio increased to 88 without a corresponding increase in the initial rate of P-700 photooxidation. Thus, the quantum efficiency of P-700 photooxidation dropped considerably. Apparently, chlorophyll bound to the particles by the second reconstitution process does not serve as an effective antenna for the PS I reaction center. This suggests that P-700-enriched particles have a limited number of binding sites for antenna chlorophyll. Excess chlorophyll introduced by the second reconstitution might have been randomly absorbed on the particles.

Chl *a* purified from the ether extracts could also combine with P-700-enriched particles. However, the quantum efficiency of P-700 photooxidation was significantly lower than that in the particles reconstituted with the ether extracts. Since the ether extracts contain other lipids besides chlorophyll, possible effects of several lipids on reconstitution with Chl *a* were examined. Combined addition of Chl *a* and monogalactosyldiacylglycerol or phosphatidylcholine increased the quantum yield of P-700 photooxidation to a high level, comparable to that obtained by using the ether extracts. For some unknown reason, phosphatidylethanolamine apparently suppresses the association of chlorophyll with P-700-enriched particles.

Absorption spectra

The absorption spectra of PS I particles and

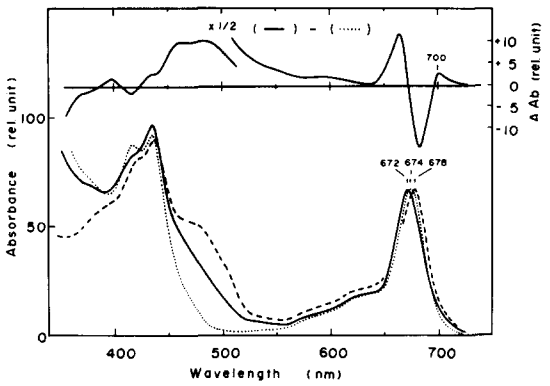

Fig. 1. Absorption spectra of PS I particles (- - - - - -), P-700-enriched particles (·····) and the particles reconstituted with ether extracts (———). They were normalized at their α-band maxima. P-700 was present in the oxidized state in all samples. Chlorophyll contents in P-700-enriched particles and the reconstituted particles were 4.5 and 13.4%, respectively, of that in the PS I particles. The upper part shows the difference spectrum obtained by subtracting the absorption spectrum of P-700-enriched particles from that of the reconstituted particles. For other details, see Table I.

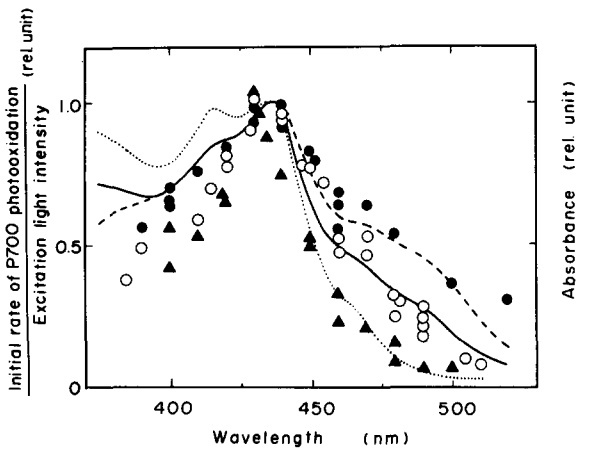

Fig. 2. Action spectra for P-700 photooxidation and absorption spectra of PS I particles (● and - - - - - -), P-700-enriched particles (▲ and ·····) and the particles reconstituted with ether extracts (○ and ———). All curves were normalized at 435 nm. Chlorophyll contents in the reconstituted particles and P-700-enriched particles were the same as described in Fig. 1. Monochromatic excitation light was supplied from a 1000 W Xe lamp equipped with a Nikon G-250 grating monochrometer (half-band width, approx. 9 nm), an infrared-absorbing filter (Hoya HA-50) and a 10 cm thick water layer. P-700 photooxidation was measured at 697 nm with a reference wavelength at 730 nm, in the presence of 5 mM ascorbate.

P-700-enriched particles before and after reconstitution with ether-extracted pigments are shown in Fig. 1. PS I particles showed the red-band maximum at 678 nm, whereas the P-700-enriched particles had a maximum at 674 nm. The reconstituted particles showed a peak at even shorter wavelength (670–672 nm). When the reconstitution process was repeated once more, the absorption peak shifted to 669 nm. Note that the reconstitution also increased absorbance around 700 nm. A small positive peak at 700 nm is seen in the difference spectrum obtained by subtracting the absorption spectrum of P-700-enriched particles from that of the reconstituted particles (see upper part of Fig. 1). The absorption spectrum of the particles reconstituted with Chl *a* (with or without lipid) was essentially similar to the solid-line spectrum in Fig. 1, except between 450 and 500 nm where absorption bands of carotenoids or Chl *b* appear. The particles reconstituted with ether-extracted pigments showed a significant absorption in this wavelength region and the difference spectrum indicates the reassociation of carotenoids with the particles. This prompted us to measure the action spectra of P-700 photooxidation in this wavelength region in three different particles. The results are presented in Fig. 2. Agreements between the action and the corresponding absorption spectra are evident. This strongly suggests that carotenoids reincorporated into the P-700-enriched particles also serve as the effective light-harvesting pigments of PS I.

Fluorescence properties

Fig. 3 shows the fluorescence emission spectra determined at −196°C. It has been previously described that P-700-enriched particles show a light-induced rise in the fluorescence yield on illumination in the presence of reductants, such as ascorbate or dithionite, and that the constant and variable fluorescence determined at −196°C has different emission spectra with peaks at 682 and 698 nm, respectively [5]. The dotted line in Fig. 3 shows a typical emission spectrum of the constant fluorescence in P-700-enriched particles determined without any reductants. On reconstitution with ether extracts the 682 nm band disappeared and a large band appeared at around 730 nm (solid line in Fig. 3), which is quite similar to

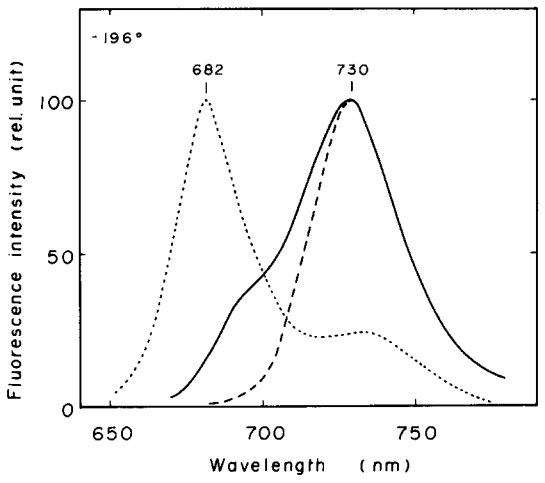

Fig. 3. Fluorescence emission spectra PS I particles (- - - - -), P-700-enriched particles (· · · · · ·) and particles reconstituted with ether extracts (———) at liquid nitrogen temperature. P-700 was present in the oxidized state in all samples. The spectra were normalized at their main peaks. Chlorophyll contents in the reconstituted particles and P-700-enriched particles were 18 and 4%, respectively, of that in the PS I particles. The sample was placed in a cell of 1 mm thickness and cooled to −196°C with liquid nitrogen. The blue excitation light used was the same as described in Table I. The light intensity determined with a Kipp and Zonen thermopile was 1000 erg·cm^{-2}·s^{-1}. Fluorescence emitted on the same side as the illumination was analyzed with a Nikon G-250 grating monochrometer (half-band width, 9 nm) and detected by a photomultiplier (Hamamatsu TV, R663). The emission spectra were not corrected for the spectral sensitivity of the photomultiplier.

that of PS I particles (dashed line in Fig. 3). Thus, the reconstitution of antenna pigments in P-700-enriched particles was accompanied by the regeneration of the far-red fluorescence peak, a characteristic feature of the pigment system of PS I. This fluorescence band may be related to the 700 nm absorption band which had been diminished in P-700-enriched particles but enhanced in the reconstituted particles (see Fig. 1). The absence of the fluorescence peak around 680 nm which is emitted from the antenna chlorophyll not immediately adjacent to the reaction center of photosystem-I [5] suggests that a form of Chl *a* showing an absorption maximum at 700 nm, 'Chl *a* 700', acts as a good energy sink in the antenna of PS I. Butler [9] has identified the far-red fluorescence emitter as an energy-sink chlorophyll, 'C705'. The reconstituted 700 nm band may correspond to C705.

The PS I particles showed, even at room temperature, a prominent emission at 720 nm, in addition to a main emission band at 687 nm (Fig. 4). The far-red emision band largely disappeared in P-700-enriched particles and the main band shifted to 679 nm. The reconstitution with pigments brought the emission spectrum of P-700-enriched particles again closer to that of the PS I particles. The main emission band occurred at 683 nm and an additional far-red band appeared.

Table II presents the relative yields of fluorescence emitted from the PS I particles, the P-700-enriched particles and the particles reconstituted with ether extracts. At room temperature, P-700-enriched particles were much more fluorescent than PS I particles. The recombination with pigments lowered the fluorescence yield to a level close to that of the PS I particles. This may be attributed to different degrees of competition between fluorescent (radiative) and heat (nonradiative) degradation of the excited chlorophyll. The absence of Chl *a* 700 might be responsible for a high radiative degradation in P-700-enriched particles.

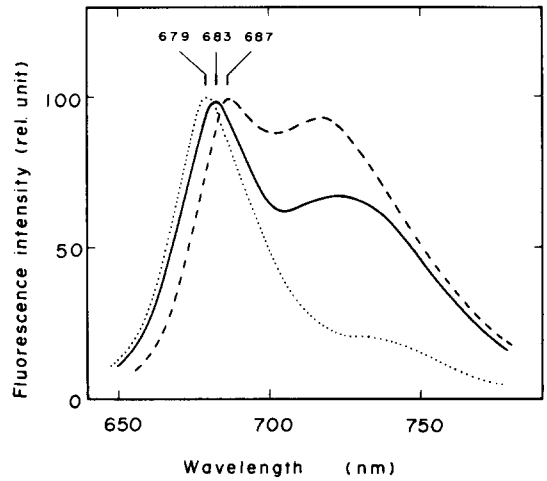

Fig. 4. Fluorescence emission spectra of PS I particles (- - - - -), P-700-enriched particles (· · · · · ·) and particles reconstituted with ether extracts (———) at room temperature. The spectra were normalized at their main emission peaks. Chlorophyll contents in the reconstituted particles and P-700-enriched particles were 16 and 5%, respectively, of that in PS I particles. For other details, see Fig. 3.

TABLE II

THE RELATIVE YIELDS OF THE FLUORESCENCE EMITTED FROM PS I PARTICLES, P-700-ENRICHED PARTICLES AND THE RECONSTITUTED PARTICLES

The total fluorescence yields were obtained by the integration of the emission spectra in Figs. 3 and 4, and normalized with the chlorophyll concentration. See Figs. 3 and 4 for other details.

Fractions	Relative fluorescence yields (%)	
	Room temperature	−196°C
PS I particles	100	100
P-700-enriched particles	540	57
Reconstituted particles	126	170

In contrast, fluorescence yields of PS I particles and the reconstituted particles were higher than that of P-700-enriched particles at liquid nitrogen temperature. This may be related to the observation that the far-red emission band was predominant both in PS I particles and in the reconstituted particles but not in P-700-enriched particles at low temperatures (Figs. 3 and 4). Thus, relative fluorescence yield will be reversed as the far-red fluorescence band was more strongly intensified than the 680 nm band with lowering temperature. Evidence has also been reported by others that the yield of the far-red emission band increased more strongly at low temperatures than the PS II fluorescence [10–12].

The far-red fluorescence band is present in purified PS I reaction center with the Chl *a*/P-700 ratios being over 70 [13–15], whereas the P-700-Chl *a*-protein complexes isolated with Triton X-100 and containing about 40 Chl *a*/P-700 [15–20] have no prominent emission at longer wavelength. The present work showed that reconstructed particles with a Chl *a*/P-700 ratio of about 30 exhibited the far-red fluorescence band and suggests that ether or Triton X-100 probably destroys the emitter of the far-red band, a special form of Chl *a* having an absorption maximum at about 700 nm.

Acknowledgements

The author wishes to thank Professor S. Katoh (Department of Pure and Applied Sciences, College of General Education), University of Tokyo, for support and advice during the work and preparation of the manuscript. Thanks are also due to Dr. B. Ke, C.F. Kettering Research Laboratory, for reading and commenting on the manuscript. This work was supported in part by a Grant-in-Aid for Scientific Research from The Ministry of Education, Science and Culture, Japan.

References

1 Kok, B. (1961) Biochim. Biophys. Acta 48, 527–533
2 Sane, P.V. and Park, R.B. (1970) Biochem. Biophys. Res. Commun. 41, 206–210
3 Hirayama, O., Matsuda, H., Senzaki, K. and Masuda, T. (1979) Agric. Biol. Chem. 43, 1205–1210
4 Ikegami, I. and Katoh, S. (1975) Biochim. Biophys. Acta 376, 588–592
5 Ikegami, I. (1976) Biochim. Biophys. Acta 449, 245–258
6 Strain, H.H., Cope, B.T. and Svec, W. (1971) Methods Enzymol. 23, 452–476
7 Allen, C.F. and Good, P. (1971) Methods Enzymol. 23, 523–547
8 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
9 Butler, W.L. (1961) Arch. Biochem. Biophys. 93, 413–422
10 Boardman, N.K., Thorne, S.W. and Anderson, J.M. (1966) Proc. Natl. Acad. Sci. U.S.A. 56, 586–593
11 Searle, G.F.W., Barber, J., Harris, L., Porter, G. and Tredwell, C.J. (1977) Biochim. Biophys. Acta 459, 390–401
12 Butler, W.L., Tredwell, C.J., Malkin, R. and Barber, J. (1979) Biochim. Biophys. Acta 545, 309–315
13 Anderson, J.M., Waldron, J.C. and Thorne, S.W. (1978) FEBS Lett. 92, 227–233
14 Satoh, K. (1979) Plant Cell Physiol. 20, 499–512
15 Mullet, J.E., Burke, J.J. and Arntzen, C.J. (1980) Plant Physiol. 65, 814–822
16 Ogawa, T. and Vernon, L.P. (1969) Biochim. Biophys. Acta 197, 292–301
17 Mohanty, P., Braun, B.Z., Govindjee and Thornber, J.P. (1972) Plant Cell Physiol. 13, 81–91
18 Shiozawa, J.A., Alberte, R.S. and Thornber, J.P. (1974) Arch. Biochem. Biophys. 165, 388–397
19 Vacek, K., Wong, D. and Govindjee (1977) Photochem. Photobiol. 26, 269–276
20 Rijgersberg, C.P., Amesz, J., Thielen, A.P.G.M. and Swager, J.A. (1979) Biochim. Biophys. Acta 545, 473–482
21 Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160–171