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Antenna chlorophyll *a* has a structural role in stabilizing the functional conformation of P-700-chlorophyll-protein complexes

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The heat-stability of P-700 photooxidation in Photosystem I particles from the thermophilic cyanobacterium *Synechococcus elongatus* dramatically decreased when bulk antenna chlorophyll *a* was extracted by diethyl ether treatment. The loss of the heat stability was related to the amount of extracted antenna chlorophyll *a* because the P-700 photooxidation became increasingly sensitive to heat as the chlorophyll *a* to P-700 ratio decreased. The heat-stability of P-700 photooxidation was substantially restored on addition of chlorophyll *a*, together with a lipid, to chlorophyll-depleted particles. Phosphatidylglycerol was most effective, followed by monogalactosyldiacylglycerol, whereas phosphatidylcholine, phosphatidylinositol and digalactosyldiacylglycerol had only minor effects. Addition of either chlorophyll *a* or lipid alone was without effect. A part of chlorophyll *a* rebound to the particles served as antenna of P-700 photooxidation and there was a good correlation between extents of the stabilization and increases in the antenna size. Thus, the stabilization of the P-700 photoresponse is ascribed to chlorophyll *a* molecules bound to the functional binding sites. The heat stability of P-700 photooxidation in Photosystem I particles from spinach was also largely lost by ether-treatment and restored by readdition of chlorophyll *a* and a lipid such as phosphatidylglycerol. It is concluded that antenna chlorophyll *a* has an important structural role in stabilizing P-700-chlorophyll-protein complexes.

Introduction

P-700 is one of the most heat-stable functional components in photosynthetic electron transport chain in plants and cyanobacteria. The half-inactivation of P-700 activity is achieved by heat treatment of PS I preparations from higher plants at about 70°C for 5 min [1,2]. In particular, P-700 is extremely stable to heat in the thermophilic cyanobacterium *Synechococcus elongatus*. Koike et al. [3] showed that P-700 was only partly inactivated by treatment of the cyanobacterial thylakoids at 100°C for 5 min. The heat-stability of P-700 in the cyanobacterium decreased when PS I preparations were isolated with detergents but about a half of P-700 still survived heat treatment at 93°C for 5 min [4].

We previously showed that treatment of digitonin-spinach PS I particles with diethyl ether containing an appropriate amount of water results in extraction of most antenna chlorophyll without any significant loss of P-700 [5]. The ether-washed preparations, which are enriched in P-700 relative to antenna pigments, have served as excellent materials for investigation of the antenna and electron acceptors of PS I [6–12]. Recently, the experiments were extended to *Synechococcus elongatus* and PS I particles having the chlorophyll *a* to P-700 ratios of 13 were prepared and characterized [13].

In the present work, we report evidence that the heat stability of P-700 is supported by antenna chlorophyll *a* molecules bound to PS I particles both in *Synechococcus* and in spinach. P-700 became sensitive to heat when bulk antenna chlorophyll *a* had been removed with ether and the heat-stability of P-700 was substantially restored on readdition of chlorophyll *a*,

Abbreviations: Chl, chlorophyll; PS, Photosystem; PG, phosphatidylglycerol; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; PC, phosphatidylcholine; PI, phosphatidylinositol.

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together with a lipid, to chlorophyll-*a*-depleted particles. Evidence is also presented indicating that only chlorophyll *a* molecules bound to the functional binding sites contribute to the stabilization of P-700.

Materials and Methods

Procedures for preparation of *Synechococcus* and spinach PS I particles having various chlorophyll *a*/P-700 ratios were described previously [5,13]. *Synechococcus* thylakoid membranes or digitonin-PS I preparations from spinach were extracted twice with diethyl ether that contained various amounts of water. PS I particles were solubilized with 0.1–0.3% Triton X-100 at 4°C for 30 min and recovered by centrifugation [5,13].

Chlorophyll *a*-depleted PS I particles were reconstituted with the ether extract, or chlorophyll *a* with and without a lipid, by the method described previously [7]. The particles which had originally contained about 200 μg chlorophyll *a* before ether extraction, were suspended in 1 ml of 20 mM phosphate buffer (pH 8) and 10–20 μl of ethanol solution of the ether extract or chlorophyll *a* plus a lipid was added to the suspensions with stirring. After incubation for 30 min at 4°C, the reconstituted particles were collected by centrifugation.

P-700 was determined by measuring light-induced absorption changes at 696 nm with a Hitachi 556 dual-wavelength spectrophotometer as described previously [7–11]. PS I particles were suspended in 20 mM phosphate buffer (pH 8), 50 mM NaCl, 5 mM ascorbate, 3 μM dichlorophenolindophenol and 20 μM vitamin K-3. The blue excitation light with the maximum intensity at 430 nm was obtained from a 100 W halogen lamp through an infrared-absorbing filter (Hoya HA-50), two blue filters (Hoya B-460 and Corning 5-60) and a 5 cm water layer. P-700 was also quantified by measuring the ferricyanide-oxidized minus ascorbate-reduced difference spectrum. The extinction coefficient of P-700 was assumed as 64 mM^{-1} cm^{-1} [14]. All measurements were carried out at 5°C. Chlorophyll concentration was determined according to the method of Arnon [15].

Monogalactosyldiacylglycerol and digalactosyldiacylglycerol from spinach were purchased from Hunakoshi Co. Chlorophyll *a* from spinach, and phosphatidylglycerol, phosphatidylcholine and phosphatidylinositol from soybean were obtained from Wako Chemicals.

Results

Fig. 1 shows the heat-stability of P-700 photooxidation in three preparations of *Synechococcus* PS I particles having different chlorophyll *a*/P-700 ratios. The particles were prepared from the thylakoid membranes which had been washed with ether containing different amounts of water [13]. Magnitudes of the photoresponse were determined after treatment of the preparations at indicated temperatures for 5 min. Because *Synechococcus* particles prepared from ether-washed membranes are depleted of vitamin K-1 [13], P-700 photooxidation was determined in the presence of 20 μM vitamin K-3. The results shown in Fig. 1 clearly demonstrate that the P-700 photoresponse became increasingly sensitive to heat as the chlorophyll *a*/P-700 ratio decreased. The magnitude of the photoresponse was not decreased by heat treatment lower than 60°C for 5 min in ether-extracted particles having a chlorophyll *a*/P-700 ratio of 54. On the other hand, about a half of P-700 was inactivated by heat treatment at 50°C for 5 min in the particles having a chlorophyll *a*/P-700 ratio of 22. Decrease of the ratio to 13 was associated with further decline of the heat-stability of P-700 showing the half-inactivation of P-700 by heat treatment at 35°C for 5 min, which is about 60°C lower than that in PS I complexes isolated from untreated membranes [3]. Chemically oxidized-minus-reduced difference spectra of P-700 revealed that the inactivation of P-700 photooxidation was due to the destruction of P-700 itself (data not shown). This indicates that amounts of P-700 determined in the presence of vitamin K-3 were independent of inactivation of the iron-sulfur centers (see Refs. 3, 4). The same results were obtained by extraction of antenna chlorophyll *a* from spinach PS I particles. Table I shows that the half-inactivation temperature of the photoresponse decreased with decreasing ratio of chlorophyll *a* to P-700. Although spinach were generally heat-labile than cyanobacteria, P-700 photooxidation in spinach PS I particles with a chlorophyll *a*/P-700 ratio of 8 was as sensitive to heat as that in

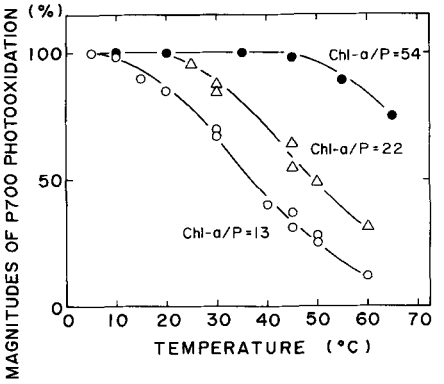

Fig. 1. Heat stability of P-700 photooxidation in *Synechococcus* PS I particles having different chlorophyll *a* to P-700 ratios. PS I particles with the ratio of 54 (solid circles), 22 (open triangles) and 13(open circles) were prepared from the thylakoid membranes which had been washed with ether containing water at 0, 50 and 100% saturation levels, respectively. The samples were incubated for 5 min at the indicated temperatures, rapidly cooled and then P-700 photooxidation was determined at 696 nm in the presence of 5 mM ascorbate, 3 μM dichlorophenolindophenol and 20 μM vitamin K-3.

TABLE I

Effects of ether wash on heat-stability of P-700 photooxidation in spinach PS I particles

Samples were treated at various temperatures for 5 min and P-700 photooxidation was determined in the presence of 1 mM methyl viologen. The temperatures at which the magnitude of P-700 photooxidation was decreased to a half of that obtained before heat treatment are presented in table. For other experimental conditions, see Fig. 1.

Preparations	Chl *a*/P-700 (mol/mol)	Half-inactivation temperatures (°C)
PS I particles	148	80
Ether-washed PS I particles	56	65
	24	54
	8	32

Synechococcus particles with the ratio of 13. Thus, irrespective of large differences in the heat-stability of proteins between the two organisms, the primary photochemistry of PS I became similarly unstable after extraction of antenna chlorophyll *a*.

In the above and following experiments, the 5 min period of heat treatment was employed to assess stability of P-700. The rationale is that, as shown in Fig. 2, time-courses of inactivation were apparently biphasic, consisting of an initial rapid phase and a subsequent slow phase, and the initial rapid inactivation completed within the first 5 min of the incubation. Thus, PS I is heterogeneous in chlorophyll-*a*-depleted particles and

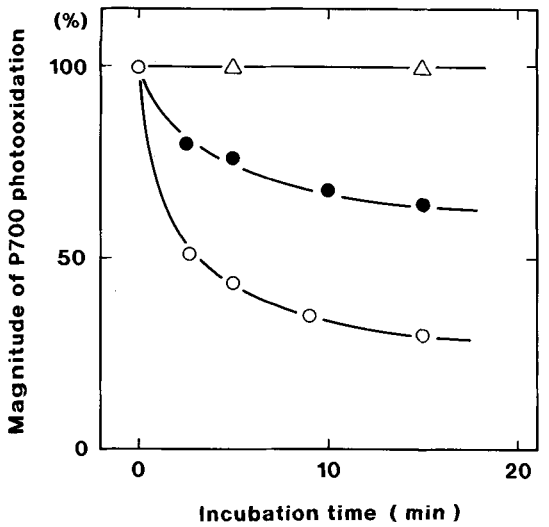

Fig. 2. Time-courses of heat inactivation of P-700 photooxidation in spinach preparations. Samples were incubated at 40°C for indicated periods of time and magnitudes of P-700 photooxidation were determined as described in Fig. 1. Open triangles, spinach thylakoid membranes; open circles, chlorophyll-*a*-depleted PS I particles with chlorophyll *a* to P-700 ratio of 10; solid circles, the same particles reconstituted with 50 μg chlorophyll *a* and 100 μg PG (chlorophyll *a* to P-700 ratio, 25).

TABLE II

Effects of reconstitution with chlorophyll a and lipids on the heat-stability and initial rate of P-700 photooxidation in chlorophyll a-depleted Synechococcus PS I particles

Synechococcus PS I particles having a chlorophyll *a*/P-700 ratio of 13 were reconstituted with the ether extract containing 90 μg chlorophyll *a*, or with 50 μg chlorophyll *a* and/or 200 μg lipid. Percentage inactivation of P-700 photooxidation was determined by measuring magnitudes of light-induced absorption changes at 696 nm before and after treatment of PS I particles at 50°C for 5 min. The initial rates of P-700 photooxidation were measured before heat treatment with low intensities of light (500 to 2000 erg/cm^2 per s) where the rate of photooxidation was proportional to light intensity. The relative rates corrected for the excitation light intensities are presented, taking the value of non-reconstituted particles as 100%.

Preparations	Chl *a*/P-700 (mol/mol)	P-700 photooxidation	
		Heat inactivation (%)	Initial rate (%)
Thylakoid membranes	147	0	-
Chl-*a*-depleted PS I particles	13	73	100
Reconstituted with:			
ether extract	90	55	150
Chl *a*	40	74	98
PG	13	71	101
Chl *a* + PG	65	25	203
Chl *a* + MGDG	64	44	162
Chl *a* + PI	68	60	123
Chl *a* + PC	30	65	100
Chl *a* + DGDG	36	68	111

we mainly monitored inactivation of P-700 in a heat-labile part of PS I.

The above observations strongly suggest that antenna chlorophyll *a* contributes to stabilization of PS I reaction-center complexes. This suggestion was supported by experiments, in which chlorophyll *a*-depleted particles were reconstituted with the ether-extract or chlorophyll *a* (Table II). When the ether extract containing 90 μg chlorophyll *a* (i.e., about half of the total ether extract) had been added back to *Synechococcus* particles prepared from ether-washed membranes, the percentage of P-700 that survived by the 5 min treatment at 50°C increased from 27% to 45%. The chlorophyll *a*/P-700 ratio of the particles, which had been separated by centrifugation from the supernatant containing free chlorophyll *a*, also increased from 13 to 90. The results suggest that P-700 is stabilized by rebinding of chlorophyll *a* to the PS I particles. No further improvement of the heat stability was observed on addition of larger amounts of the ether extract (data not shown).

Unexpectedly, addition of purified chlorophyll *a* alone had essentially no effect on the heat stability of P-700, although the chlorophyll *a*/P-700 ratio increased to 40. The ether extract also contained lipids,

but addition of any single lipid species failed to improve the heat stability. However, P-700 was appreciably stabilized by the combined addition of chlorophyll *a* and a lipid. The most effective lipid was PG, which, when added together with chlorophyll *a*, decreased the extent of P-700 destruction at 50°C from 73% to 25%. Essentially the same stabilizing effects were obtained whether the lipid and chlorophyll *a* were added simultaneously or chlorophyll *a* was added back to the particles which had been reconstituted with the lipid (data not shown). MGDG plus chlorophyll *a* also protected more than half of P-700 from heat inactivation, but PI plus chlorophyll *a* showed a smaller effect. Note that, irrespective of the degree of stabilization, the three lipids promoted the binding of chlorophyll *a* to similar extents. Thus, there is no correlation between the degree of stabilization and the amount of rebound chlorophyll *a*. PC and DGDG were less effective both in the binding of chlorophyll *a* and in the protection of the P-700 photoresponse.

We showed previously that chlorophyll *a* reincorporated into the ether-washed PS I particles from spinach serves as an efficient antenna, provided that a lipid was simultaneously added [7]. Experiments were carried out to examine whether chlorophyll *a* molecules bound to *Synechococcus* particles functions as antenna pigments. As a measure of the functional antenna size of PS I, the initial rate of P-700 photooxidation was determined with a limiting intensity of light, assuming that the quantum efficiency of the PS I photochemistry is not affected by reconstitution of chlorophyll *a* and a lipid. Table II shows that addition of the ether extract increased the initial rate by 50%. Whereas addition of chlorophyll *a* or PG alone had no effect, chlorophyll *a* plus PG doubled the initial rate of P-700 photooxidation. When added together with chlorophyll *a*, MGDG accelerated P-700 photooxidation by more than 50% and PI in a lesser extent. As stated above, these three lipids were similarly effective in promoting rebinding of chlorophyll *a*. Thus, the results indicate that not all the chlorophyll *a* molecules bound to, or coprecipitated with, PS I particles can transfer excitation energy to P-700 and degree of the functional reconstitution varies depending upon lipid species added. PC and DGDG showed no or only small effects. Thus, there was a good correlation between extents of the stabilization and increases in the antenna size. It is concluded, therefore, that only chlorophyll *a* molecules bound to the functional sites contribute to the stabilization of P-700.

In the above experiments, the amount of chlorophyll *a* added was 50 μg, which corresponds to about a quarter of the total chlorophyll *a* originally present in the thylakoid membranes employed for preparation of PS I particles. Addition of larger amounts of chlorophyll *a* increased in the amount of chlorophyll *a* rebound to, or coprecipitated with, the particles without any further increase in the antenna size or in the heat-stability of P-700. This suggests that ether-extracted particles have only a limited number of functional binding sites for chlorophyll *a* and the pigment molecules added in excess unspecifically bind to, or are adsorbed on, the surface of the particles (cf. Ref. 7). In fact, the absorption spectrum of the particles reconstituted with an excess amount of chlorophyll *a* showed a significant shoulder at 700–730 nm, which is indicative of aggregation of chlorophyll *a* molecules (data not shown).

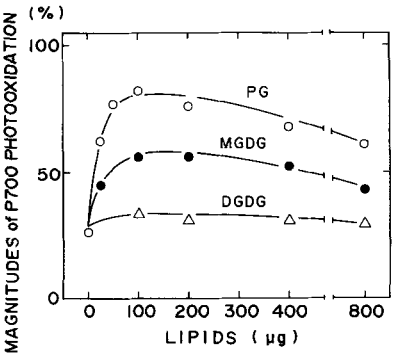

Fig. 3. Heat stability of P-700 photooxidation in *Synechococcus* particles reconstituted with 50 μg chlorophyll *a* and varied amounts of PG, MGDG or DGDG. The chlorophyll *a*/P-700 ratio of the particles was 13 before reconstitution. Magnitudes of P-700 photooxidation in the particles treated at 50°C for 5 min were determined relative to those of the photoresponse obtained before heat treatment. For other experimental conditions, see Fig. 1 and Table II.

Fig. 3 shows the heat stability of P-700 in chlorophyll *a*-depleted *Synechococcus* particles which were reconstituted with a fixed amount of chlorophyll *a* and varied amounts of PG, MGDG or DGDG. At all the concentrations of lipids examined, PG was most effective in stabilizing P-700 photooxidation, followed by MGDG, and DGDG was the least effective. The maximum recovery of the heat stability was observed on addition of 100 μg PG or MGDG, which is roughly comparable to the amount of PG or half that of MGDG originally present in the thylakoid membranes from which the particles had been isolated. PG and MGDG became less effective as their concentrations were raised above 200 μg. The fluorescence yield of chlorophyll *a* also increased at high concentrations of the lipids, indicating increased distribution of free chlorophyll *a* in liposomes or micelles of the lipids added.

P-700 photooxidation in ether-washed spinach particles was also stabilized by chlorophyll *a* and a lipid (Table III). Combined addition of chlorophyll *a* and PG was again most effective both in restoring the heat stability of P-700 and in accelerating the initial rate of P-700 photooxidation. The reconstitution preferentially diminished the initial rapid inactivation phase (see Fig.

TABLE III

Effects of reconstitution with ether extract, or with chlorophyll a and lipids on the heat-stability and initial rate of P-700 photooxidation in chlorophyll-a-depleted spinach PS I particles

Spinach PS I particles with the chlorophyll *a*/P-700 ratio of 8 were reconstituted with the ether extract containing 50 μg chlorophyll *a*, or with 50 μg chlorophyll *a* plus 200 μg PG or 200 μg MGDG. Heat inactivation of P-700 photooxidation was determined by measuring magnitudes of the photoresponse before and after treatment of samples at 40°C for 5 min. For other experimental conditions, see Table II.

Preparations	Chl *a*/P-700 (mol/mol)	P-700 photooxidation	
		Heat inactivation (%)	Initial rate (%)
PS I particles	145	0	–
Chl-*a*-depleted PS I particles	8	68	100
Reconstituted with ether extract	43	55	162
Chl *a* + PG	23	23	225
Chl *a* + MGDG	30	52	169

2). The results presented in Table III also revealed a notable feature of stabilization. The initial rate was accelerated by 125% as the chlorophyll *a* to P-700 ratio increased from 8 to 23. Thus, the number of chlorophyll *a* molecules incorporated into the functional sites in the presence of PG appears to be about 13 per P-700. The percentage of P-700 inactivated at 40°C also decreased from 68 to 23%. Thus, P-700 is strongly stabilized by binding of a relatively small number of chlorophyll *a* molecules.

Discussion

The core of the PS I reaction-center complex consists of the two Chl-binding subunit proteins of about 83 kDa (psaA and psaB gene products), each of which carries several tens of chlorophyll *a* molecules (cf. Ref. 16). There are two populations of the antenna chlorophyll *a* molecules which are different in terms of the binding affinity. A major population of chlorophyll *a* is extractable with wet ether, whereas a minor population of 8–13 chlorophyll *a* per P-700 is resistant to the ether extraction [5,13]. The minor population of chlorophyll *a* appears to be closely associated around P-700 because its extraction with ether at high saturation level of water resulted in a parallel decrease in the P-700 content [5,13]. The major population of ether-extractable chlorophyll *a* molecules is considered to serve only as light-harvesting pigments because the ether treatment did not affect the primary photochemistry of PS I [5–13].

The present work demonstrates a novel function of the major population of antenna chlorophyll *a*, i.e., stabilization of the functional molecular organization of P-700. The heat-stability of P-700 in both *Synechococcus* and spinach PS I preparations decreased as chlorophyll *a*/P-700 ratio was reduced by selective extraction of antenna chlorophyll *a* with wet ether, but restored on addition of the ether extract or chlorophyll *a* plus a lipid to the chlorophyll-*a*-depleted particles. Addition of chlorophyll *a* alone was totally ineffective and there were no correlation between extents of the stabilization and amounts of the total chlorophyll *a* rebound to the particles. However, the restoration of the heat-stability can be well related to the fraction of rebound chlorophyll *a* molecules which serves as antenna pigments of PS I. The results suggest that P-700 is stabilized by chlorophyll *a* molecules which rebind to their original binding sites. The role of lipids appears to assist the reincorporation of chlorophyll *a* into the binding sites. Lipids may also have an additional stabilizing effects on the PS I particles reconstituted with chlorophyll *a*.

Because P-700 is chlorophyll *a* molecules with a dimeric organization that locate between the two large subunit proteins of the PS I reaction-center complexes (see Ref. 16), the heat stability of P-700 can be ascribed to the solid conformation of the subunit proteins. In fact, P-700 is much more heat-stable in the thermophilic cyanobacterium, that has very stable proteins [3], than in spinach. However, stability of P-700 similarly decreased with increasing extraction of antenna pigments both in spinach and in the thermophilic *Synechococcus*. It is concluded, therefore, that antenna chlorophyll *a* molecules stabilize P-700 through stabilization of the two large subunit proteins of PS I, regardless of the large difference between the organisms.

The present work demonstrates, to our knowledge for the first time, that chlorophyll contributes to the physical stability of chlorophyll-carrying proteins. There is evidence suggesting that chlorophyll-binding proteins are also stabilized by chlorophyll against proteolytic digestion. The newly synthesized apoproteins of light-harvesting chlorophyll *a*/*b* protein of PS II were rapidly degraded in the chlorophyll-*b*-less mutants but not in the wild-type of rice [17]. A good correlation was found between the rates of protein breakdown and the extent of chlorophyll *b* deficiency, indicating that the binding of the full complement of chlorophyll *a* and *b* is needed to stabilize the apoproteins against attack by proteases. More recently, Mullet et al. [18] showed that binding of chlorophyll stabilizes the newly synthesized apoproteins of the D1 and CP43. Takabe et al. [19] showed that when PS I complexes which had been partially digested with trypsin were subjected to SDS-gel electrophoresis, the complexes migrated, in spite of numerous cuts in the polypeptide chains, as a single green band, but the complexes were totally disinte-

grated on electrophoresis under conditions where chlorophyll was solubilized. Thus, chlorophyll appears to be needed to maintain the biochemical or physical stability of chlorophyll proteins.

The three-dimensional structure of *Prosthecochloris* antenna chlorophyll protein shows that chlorophyll is an integral part of the protein [20]. Seven bacteriochlorophyll molecules present in the interior of the protein matrix are oriented with their long hydrocarbon chains towards the inside, thereby forming a strongly hydrophobic region in the center of the protein molecule. This, together with weak interactions between the head-group of chlorophyll containing Mg^{2+} and amino-acid residues such as histidines, will increase the thermodynamical stability of the protein. Antenna chlorophyll *a* molecules may similarly contribute to the stability of the two chlorophyll-binding subunits of the PS I reaction-center complex. In fact, most of histidine residues are present in the membrane spanning herical regions of the subunit polypeptides [21]. Thus, the extraction of antenna chlorophyll *a* will result in a fragile conformation of the subunit proteins, but the structural changes are reversed by rebinding of the pigment molecules to their original binding sites.

Acknowledgments

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References

1 Takamiya, K. and Nishimura, M. (1972) Plant Cell Physiol. 13, 35–47.
2 Shuvalov, (1976) Biochim. Biophys. Acta 430, 113–121.
3 Koike, H., Satoh, Ka. and Katoh, S. (1982) Plant Cell Physiol. 23, 293–299.
4 Sonoike, K., Hatanaka, H., Katoh, S. and Itoh, S. (1990) Plant Cell Physiol. 31, 865–870.
5 Ikegami, I. and Katoh, S. (1975) Biochim. Biophys. Acta 376, 588–592.
6 Ikegami, I. (1976) Biochim. Biophys. Acta 449, 245–258.
7 Ikegami, I. (1983) Biochim. Biophys. Acta 722, 492–497.
8 Itoh, S., Iwaki, M. and Ikegami, I. (1987) Biochim. Biophys. Acta 893, 508–516.
9 Ikegami, I. Setif, P. and Mathis, P.(1987) Biochim. Biophys. Acta 894, 414–422.
10 Mathis, P., Ikegami, I. and Setif, P. (1988) Photosynthesis Res. 16, 203–210.
11 Ikegami, I. and Itoh, S. (1988) Biochim. Biophys. Acta 934, 39–46.
12 Kim, D., Yoshihara, K. and Ikegami, I. (1989) Plant Cell Physiol. 30, 679–684.
13 Ikegami, I. and Katoh, S. (1989) Plant Cell Physiol. 30, 175–182.
14 Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160–171.
15 Arnon, D.I. (1949) Plant Physiol. 24, 1–15.
16 Golbeck, J. (1987) Biochim. Biophys. Acta 895, 167–204.
17 Terao, T. and Katoh, S. (1989) Plant Cell Physiol. 30, 571–580.
18 Mullet, J.E., Klein, P.G. and Klein, R.R. (1990) Proc. Natl. Acad. Sci. USA 87, 4038–4042.
19 Takabe, T., Ishikawa, H. and Kubo, S. (1986) Agricul. Biol. Chem. 50, 1131–1137.
20 Fenna, R.E. and Matthews, B.W. (1977) Brookhaven Symp. Biol. 28, 170–182.
21 Kirsch, W., Seyer, P. and Herrmann, R.G. (1986) Curr. Genet. 10, 843–855.