Articles

Laser-Flash Kinetic Analysis of the Fast Electron Transfer from Plastocyanin and Cytochrome c_6 to Photosystem I. Experimental Evidence on the Evolution of the Reaction Mechanism†

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ABSTRACT: The reaction mechanism of electron transfer from the interchangeable metalloproteins plastocyanin (Pc) and cytochrome c_6 (Cyt) to photooxidized P700 in photosystem I (PSI) has been studied by laser-flash absorption spectroscopy using a number of evolutionarily differentiated organisms such as cyanobacteria (*Anabaena* sp. PCC 7119 and *Synechocystis* sp. PCC 6803), green algae (*Monoraphidium braunii*), and higher plants (spinach). PSI reduction by Pc or Cyt shows different kinetics depending on the organism from which the photosystem and metalloproteins are isolated. According to the experimental data herein reported, three different kinetic models are proposed by assuming either an oriented collisional reaction mechanism (type I), a minimal two-step mechanism involving complex formation followed by intracomplex electron transfer (type II), or rearrangement of the reaction partners within the complex before electron transfer takes place (type III). Our findings suggest that PSI was able to first optimize its interaction with positively charged Cyt and that the evolutionary replacement of the ancestral Cyt by Pc, as well as the appearance of the fast kinetic phase in the Pc/PSI system of higher plants, would involve structural modifications in both the donor protein and PSI.

In some cyanobacteria and eukaryotic algae, the type I copper-protein plastocyanin (Pc)[1] and the class I *c*-type cytochrome c_6 (Cyt) act as alternative electron donors to photosystem I (PSI) (Golbeck & Bryant, 1991; Golbeck, 1992), depending on the relative availability of copper and iron in the culture medium (Wood, 1978; Ho & Krogmann, 1984; Sandmann, 1986). The two metalloproteins are acidic in green algae, as is Pc in higher plants, but can be either basic or acidic in cyanobacteria. Ho and Krogmann (1984) have suggested that the two evolutionarily unrelated proteins may have co-evolved in response to alterations/mutations in common reaction partners.

The interaction between Pc and PSI has been extensively studied in a wide variety of organisms (Wood & Bendall, 1975; Lien & San Pietro, 1979; Davis *et al.*, 1980; Haehnel *et al.*, 1980a; Tamura *et al.*, 1981; Takabe *et al.*, 1983; Bottin & Mathis, 1985; Hervás *et al.*, 1992). Fast kinetic experiments have revealed the existence of two different reduction phases in higher plants and some eukaryotic algae: the so-called fast kinetic phase ($t_{1/2}$ = 12 μs in spinach), which has been reported to contribute up to 40% of the total amplitude and has been interpreted as corresponding to the transfer of electrons from Pc closely bound to PSI to photooxidized P700 (Haehnel *et al.*, 1971, 1980a; Van Best & Mathis, 1978; Bottin & Mathis, 1985); and the second kinetic phase ($t_{1/2}$ = 110 μs), which would involve another reaction step associated with a second Pc binding state on the reaction center (Bottin & Mathis, 1987). This explanation, however, should be revised in light of the recent theories on conformational changes and molecular dynamics, and thus a reaction mechanism "gating" Pc when bound to PSI should not be ruled out (Gray & Malmström, 1989; Quin & Kostic, 1993). Hervás *et al.* (1992) have recently suggested that an optimal orientation for electron transfer to take place between the two oppositely charged proteins involved in these reactions in eukaryotic organisms is only achieved by an additional rearrangement occurring within an initially formed collision complex. For the reduction of PSI by Cyt, in particular, Nanba and Katoh (1983) have reported a half-time of less than 20 μs in the cyanobacterium *Synechococcus* sp., but such a fast phase has not been detected in the thermophilic cyanobacterium *Synechococcus elongatus* (Hatanaka *et al.*, 1993).

In order to provide a better understanding of the reaction mechanism of both Pc and Cyt when interacting with PSI, we have recently carried out a comparative laser-flash absorption spectroscopy study in the cyanobacterium *Synechocystis* sp. PCC 6803 (Hervás *et al.*, 1994). A variety

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[1] Abbreviations: Cyt, cytochrome c_6; k, reaction rate constant; K_A, equilibrium constant for the complex formation reaction; k_{obs}, observed pseudo-first-order rate constant; K_R, equilibrium constant for rearrangement of redox proteins within the reaction complex; Pc, plastocyanin; PSI, photosystem I; $t_{1/2}$, half-time.

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of cell-breaking methods and detergents was used to obtain different PSI preparations, but in no case could the fast reduction phase be observed. With the two donor metalloproteins, the observed rate constants presented a linear protein concentration dependence, thus suggesting that no "stabilized" transient complex with PSI was formed. These findings contrast with our own results in the green alga *Monoraphidium braunii* (Hervás *et al.*, 1992; Díaz *et al.*, 1994a) and the cyanobacterium *Anabaena* sp. PCC 7119 (Medina *et al.*, 1993), in which the formation of a transient electrostatic complex prior to electron transfer was evident.

This work is aimed to obtain more insight on the reaction mechanism of PSI reduction by Pc and Cyt, as well as to clearly establish the factors which determine the appearance of the fast phase of electron transfer to photooxidized P700 in the PSI complex. Experimental evidence on the evolution of the reaction mechanism in photosynthetic organisms is also presented, which is based on a comparative kinetic study by laser-flash absorption spectroscopy of PSI reduction by Pc and Cyt. A number of evolutionarily differentiated organisms have been used, namely, cyanobacteria such as *Anabaena* sp. PCC 7119 and *Synechocystis* sp. PCC 6803, green algae such as *Monoraphidium braunii*, and higher plants like spinach. These organisms have been choosen also taking into account the different isoelectric points of their respective metalloproteins: 4.2 in spinach Pc (Sykes, 1985), 3.6−3.75 in *Monoraphidium* Pc and Cyt (Campos *et al.*, 1993), 5.6 in *Synechocystis* Pc and Cyt (Hervás *et al.*, 1994), and close to 9 in *Anabaena* Pc and Cyt (Medina *et al.*, 1993).

MATERIALS AND METHODS

Purification of Metalloproteins. Spinach Pc was obtained according to Yocum (1982). Pc and Cyt from the green alga *Monoraphidium braunii* were purified following the methods described previously (Hervás *et al.*, 1992; Campos *et al.*, 1993). Pc and Cyt from the cyanobacterium *Anabaena* 7119 were obtained according to Medina *et al.* (1993). Pc and Cyt from *Synechocystis* 6803 were purified as recently described (Hervás *et al.*, 1993; Díaz *et al.*, 1994b). Protein concentration was determined using absorption coefficients of 25×10^3 M^{-1} cm^{-1} at 552 nm for reduced Cyt and 4.5×10^3 M^{-1} cm^{-1} at 597 nm for oxidized Pc (Hervás *et al.*, 1993; Díaz *et al.*, 1994b).

Preparation of PSI Particles. Spinach and *Monoraphidium* PSI particles were obtained with digitonin by following the method of Boardman (1971). Green algal cells were broken in a Sorvall Omnimixer using glass beads of 0.2 mm in diameter; thereafter, the procedure was exactly the same as for spinach. With both organisms, the chlorophyll/P700 ratio of the resulting PSI preparations was 230/1, approximately. PSI particles from *Anabaena* and *Synechocystis* were obtained by β-dodecyl maltoside solubilization as described by Rögner *et al.* (1990) and modified by Hervás *et al.* (1994); the chlorophyll/P700 ratio of the resulting PSI preparations was 130/1. The P700 content in PSI samples was calculated from the photoinduced absorbance changes at 820 nm using the absorption coefficient of 6.5 mM^{-1} cm^{-1} determined by Mathis and Sétif (1981). Chlorophyll concentration was determined according to Arnon (1949).

Standard Reaction Mixture. Unless otherwise noted, the standard reaction mixture contained, in a final volume of 0.3 mL, 50 mM Tricine−KOH buffer (pH 7.5), an amount of PSI-enriched particles equivalent to 0.75 mg of chlorophyll mL^{-1}, 0.1 mM methyl viologen, 2 mM sodium ascorbate, 10 mM $MgCl_2$, and 150 μM purified donor protein (either Pc or Cyt).

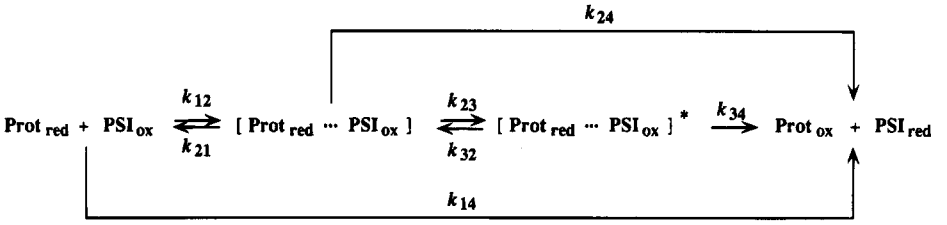
Laser-Flash Absorption Spectroscopy. Kinetics of flash-induced absorption changes in PSI were followed at 820 nm. Excitation was provided by a J. K. Lasers 2000 ruby laser (wavelength, 694 nm; pulse duration, 20−30 ns). The laser flash was attenuated with calibrated neutral density filters so as to provide a just saturating excitation energy flash. The analyzing light was provided by a 100 W tungsten−halogen lamp. The measuring detector was a silicon photodiode (Melles Griot 13DSI009). Both the reaction cell and photodiode were protected from actinic light by Oriel narrow-band glass filters with maximum transmission at 820 nm and a bandwidth of 9.4 nm. The photodiode signal output was amplified through a Melles Griot 13AMP005 amplifier (with wide bandwidth transimpedance) and recorded by a Nicolet 450 digital oscilloscope. Each kinetic trace was the average of 16−20 measurements with 30 s spacing between flashes. All experiments were carried out at room temperature (23 °C) in a 1-mm path length cuvette, which was oriented perpendicular to the laser beam and at an angle of 45° with reference to the analyzing light. The experimental setup used for measurements with *Synechocystis* metalloproteins was that described in Hervás *et al.* (1994).

Kinetic Analyses. All kinetic experiments were performed under pseudo-first-order conditions, for which the amount of donor protein was in large excess over the amount of $P700^+$. Oscilloscope traces were treated as a sum of exponential components to calculate the rate constants and amplitudes. Exponential analyses were performed using the Marquardt method with the software devised by Dr. P. Sétif. The error in the observed pseudo-first-order rate constants (k_{obs}) was estimated to be less than 10%. Kinetic analyses were carried out according to the reaction mechanisms proposed in Scheme 1. For reactions following the proposed type I or II mechanisms (see below), the rate and equilibrium constants were determined as described previously (Simondsen *et al.*, 1982). The reactions involving rearrangement of the transient complex were analyzed in sequence: First, the rate constant of the fast phase (k_{34}) was calculated; then, the equilibrium constants K_R and K_A were estimated from the percentage of fast phase according to the equation in Scheme 1.

RESULTS

P700 in PSI particles can be rapidly photooxidized by a saturating laser flash, thus inducing an almost instantaneous increase in absorbance at 820 nm. Photooxidized P700 is then reduced by the electron-donating protein, namely, Pc or Cyt (Hervás *et al.*, 1994). The absorption decay at 820 nm with PSI particles and Pc from spinach corresponds to a biphasic kinetic reaction, which can be well fitted to a double-exponential curve as described previously (Bottin & Mathis, 1985). The amplitude of the so-called fast component increases with increasing Pc concentration (see below), thus contributing up to 70% of the total absorbance change at 300 μM protein concentration (Table 1). However, the half-time of such a fast phase ($t_{1/2}$ = 11 μs) is independent of Pc concentration. These findings are in good agreement with those described previously by Bottin and Mathis (1985),

Scheme 1: Kinetic Models for the Reaction Mechanism of the Reduced Metalloproteins Cytochrome c_6 and Plastocyanin (Prot) with Photooxidized Photosystem I (PSI)[a]



$$K_A = k_{12} / k_{21}$$

$$K_R = k_{23} / k_{32}$$

$$\%\ \text{Fast Phase} = \frac{K_R K_A [\text{Donor}]}{1 + K_A [\text{Donor}] (1 + K_R)} \times 100$$

[a] The proposed models assume either an oriented collisional kinetic mechanism (type I), a minimal two-step mechanism involving complex formation followed by intracomplex electron transfer (type II), or rearrangement of the reaction partners within the complex before electron transfer takes place (type III). k, kinetic rate constant; K_A, equilibrium constant for complex formation; K_R, equilibrium constant for complex rearrangement. The asterisk denotes the reaction complex after rearrangement. See text for details.

Table 1: Kinetic Constants for PSI Reduction by Plastocyanin and Cytochrome c_6 in Evolutionarily Differentiated Organisms[a]

PSI particles	donor protein	type of mechanism	k_{14} (M^{-1} s^{-1})	k_{12} (M^{-1} s^{-1})	K_A (M^{-1})	k_{24} (s^{-1})	k_{23} (s^{-1})	K_R (M^{-1})	k_{34} (s^{-1})	amplitude[b] of fast phase (%)
spinach	sp[c] Pc	III		4.2×10^8	8.8×10^4		1.3×10^4	4.6	6.3×10^4	70
	Mb Pc	III		4.5×10^8	2.7		1.0×10^4	4.8×10^2	3.9×10^4	30
	Mb Cyt	III		2.2×10^8	4.7		1.2×10^4	1.1×10^3	3.3×10^4	55
	Ana Pc	II		1.7×10^6	4.2×10^3	0.7×10^3				
	Ana Cyt	II		1.6×10^6	8.9×10^3	0.3×10^3				
	Syn Pc	I	2.1×10^6							
	Syn Cyt	I	4.2×10^6							
Monoraphidium	sp Pc	III			1.3×10^3		1.7×10^4	1.0	4.6×10^4	<15
	Mb Pc	III (?)		3.3×10^7					nd[d]	<10
	Mb Cyt	III (?)		3.8×10^7					8.7×10^4	50
Anabaena	sp Pc	I	7.1×10^5							
	Ana Pc	I	6.5×10^7							
	Ana Cyt	III (?)		1.0×10^8					1.7×10^5	35

[a] The kinetic models were analyzed as described under Materials and Methods. The kinetic constants were obtained from plots as those in Figure 2, according to the models in Scheme 1. [b] At 300 μM donor protein concentration. [c] sp, spinach; *Mb*, *Monoraphidium braunii*; *Ana*, *Anabaena* sp. PCC 7119; *Syn*, *Synechocystis* sp. PCC 6803. [d] nd, not detectable.

even though the amplitude of the fast phase herein reported is somewhat higher.

When the kinetics of spinach PSI reduction are followed in the presence of Pc and Cyt from the green alga *Monoraphidium*, the fast phase exhibits a half-time independent of protein concentration, as in the presence of spinach Pc, but such a fast phase shows a slightly lower amplitude at 300 μM protein concentration (30% for Pc and 55% for Cyt) and a half-time of *ca.* 20 μs (Table 1). With the basic metalloproteins from the cyanobacterium *Anabaena*, as well as with the slightly acidic proteins from *Synechocystis*, no fast phase of spinach PSI reduction is detected (Figure 1, left). The observed pseudo-first-order rate constant (k_{obs}) of such monophasic kinetics can exhibit either a linear protein concentration dependence, as is the case with metalloproteins from *Synechocystis*, or a nonlinear dependence with a maximum value at saturating protein concentrations, as occurs with Cyt and Pc from *Anabaena* (see below).

According to the kinetic models proposed in Scheme 1, the redox reaction of spinach PSI with *Synechocystis* metalloproteins is consistent with a bimolecular mechanism involving formation of an undetectable transient complex, that is, with an oriented collisional kinetic model in which

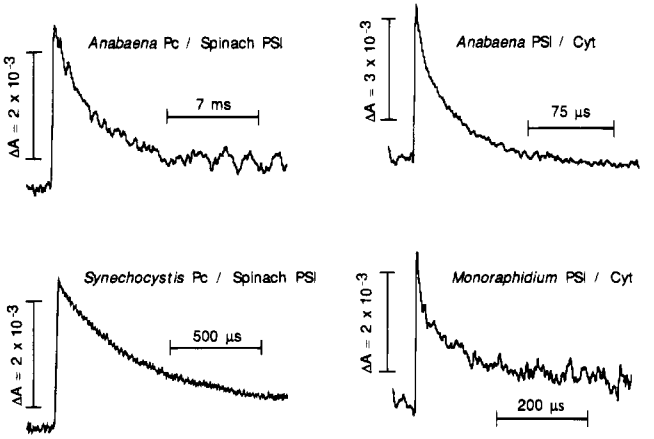


FIGURE 1: Kinetic traces of PSI reduction by plastocyanin and cytochrome c_6 from different organisms. The PSI particles were isolated either from spinach or from the same organism as were metalloproteins. In all cases, metalloprotein concentration was 250 μM, except for *Synechocystis* Pc (100 μM). The kinetic traces can be fitted to single (left) or biphasic (right) exponential curves.

k_{14} is the effective second-order rate constant of the overall redox reaction between the reduced proteins and photooxidized P700 (type I mechanism). The results with *Anabaena*

Cyt and Pc can be explained by assuming a minimal two-step kinetic mechanism involving complex formation followed by intracomplex electron transfer, as described previously (Yoneda & Holwerda, 1978; Tollin *et al.*, 1986), in which k_{12} is the second-order rate constant for the formation of the PSI/metalloprotein complex and k_{24} is the first-order rate constant of intracomplex electron transfer (type II mechanism). However, such a minimal two-step kinetic model cannot be applied as such to spinach and *Monoraphidium* metalloproteins when interacting with spinach PSI because the estimated pseudo-first-order rate constants of the slow phase at infinite donor protein concentrations do not match the observed electron transfer rate constants of the fast phase. In consequence, a third mechanism is proposed which involves rearrangement of the redox partners within the complex before electron transfer takes place (Hervás *et al.*, 1992). As can be seen in Scheme 1, k_{23} denotes the first-order rate constant of complex rearrangement, and k_{34} is the first-order rate constant of intracomplex electron transfer (type III mechanism). It should also be stated that the difference between the direct collisional mechanism (k_{14}) and the binding model without the fast phase (k_{12} and k_{24}) could also be due to a very low binding constant in the former model.

The fast phase of P700 reduction in PSI particles from *Monoraphidium* is hardly detected with Pc either from spinach or from the alga (<10−15%), but is fairly observed (up to 50%) with *Monoraphidium* Cyt (Figure 1, right; Table 1). In a simlar way, reduction of PSI particles from *Anabaena* shows no fast phase with Pc from either spinach or the cyanobacterium, but does exhibit a sharp fast phase that reaches up to 35% with *Anabaena* Cyt (Figure 1, right; Table 1). In this latter case, the fast phase runs with an electron transfer rate constant k_{34} of 1.7×10^5 s^{-1} (Table 1), that is, with a half-time of 4 μs, which is, to the best of our knowledge, the first time that such a fast kinetic phase of electron transfer to PSI in cyanobacteria is determined. As already stated for spinach PSI particles (see above), the rate constant of the fast phase in *Monoraphidium* and *Anabaena* PSI is independent of Cyt concentration, but not its amplitude.

In the kinetics of PSI reduction showing a fast phase (well fitted to a biphasic exponential curve), a second, slower phase is likewise detected (see Figure 1, right). The observed pseudo-first-order rate constant (k_{obs}) of such a slow phase, as well as that of the monophasic kinetics (see above), increases with increasing Pc or Cyt concentration. In some PSI/protein systems, the k_{obs} values for these slow phases exhibit a nonlinear protein concentration dependence, as is the case with spinach PSI in the presence of Pc or Cyt from *Anabaena* (Figure 2, lower panel) or from *Monoraphidium* (Hervás *et al.*, 1992). Notwithstanding, the observed rate constants of the slow phases depend linearly on the concentration of added proteins in other PSI/protein systems, such as those formed by the metalloproteins from *Monoraphidium* and *Anabaena* when reacting with their own PSI (Figure 2, upper panel), or by *Synechocystis* Pc and Cyt when interacting with PSI either from spinach (data not shown) or from the cyanobacterium (Hervás *et al.*, 1994). Such a linear protein concentration dependence does not allow us to clearly discriminate between the type II and III mechanisms for *Monoraphidium* and *Anabaena* PSI, even though the reaction kinetics of these two photosystems clearly exhibit a biphasic

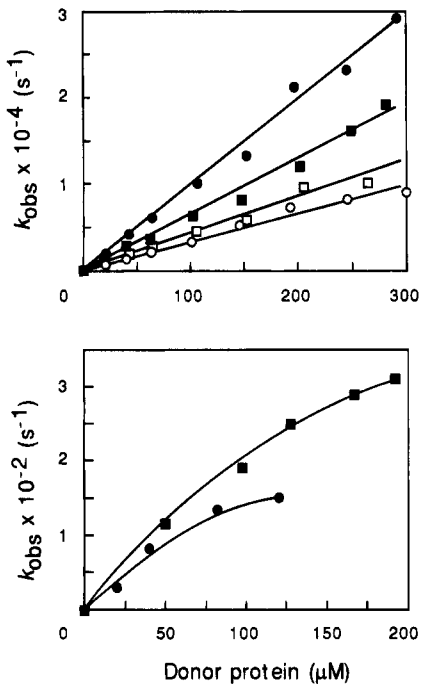


FIGURE 2: Dependence upon donor protein concentration of the observed rate constant (k_{obs}) for the slow phase of PSI reduction. Cytochrome c_6 (●, ○) and plastocyanin (■, □) were from either *Monoraphidium* (open symbols) or *Anabaena* (closed symbols). The PSI particles were isolated either from the same organism as were metalloproteins (top panel) or from spinach (bottom panel).

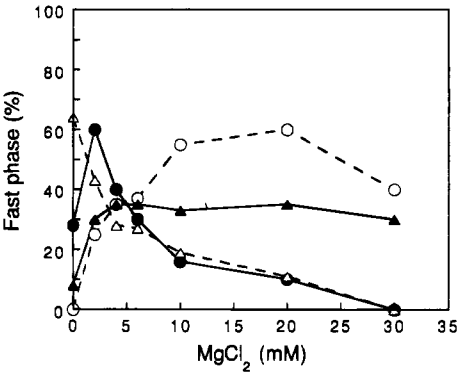


FIGURE 3: Effect of magnesium chloride concentration on the amplitude of the fast phase. The systems used were spinach plastocyanin and spinach PSI (○), *Monoraphidium* plastocyanin and spinach PSI (●), *Monoraphidium* cytochrome c_6 and *Monoraphidium* PSI (▲), and *Anabaena* cytochrome c_6 and *Anabaena* PSI (△).

profile upon photooxidation by the donor proteins isolated from the same organism (see Table 1).

The effect of electrostatic interactions on PSI reduction was investigated by determining the rate constants at varying ionic strengths, that is, at varying NaCl and/or $MgCl_2$ concentrations. Interestingly enough is the fact that no fast phase could be detected with spinach Pc and PSI in the absence of Mg^{2+} cations at pH 7.5, even at high NaCl concentrations. This suggests that Mg^{2+} cations must play a specific role in PSI−metalloprotein interactions, as previously proposed when studying the slower phase of PSI reduction in spinach and other organisms (Haehnel *et al.*, 1980b; Hervás *et al.*, 1992; Medina *et al.*, 1993; Díaz *et al.*, 1994a). Figure 3 shows that the effect of Mg^{2+} cations on the fast phase amplitude differs in the PSI/protein systems studied from all others. Thus, the fast phase amplitude

drastically decreases with increasing Mg^{2+} concentration in *Anabaena*, increases up to reach a plateau in *Monoraphidium*, and exhibits a broad bell-shapped dependence in spinach. In addition, clearly biphasic dependences are observed with spinach PSI and *Monoraphidium* Pc: The amplitude of the fast phase first increases drastically with increasing Mg^{2+} concentration, reaches a maximum value at 2−3 mM $MgCl_2$, and decreases at higher concentrations (Figure 3).

DISCUSSION

The comparative kinetic analysis herein reported using a number of evolutionarily differentiated organisms has allowed us to infer interesting conclusions on the fast phase of electron transfer to PSI. In spinach PSI particles, the fast phase is observed only with the acid donor proteins from higher plants and green algae, but not with basic (or mildly acidic) Pc and Cyt from cyanobacteria. The different lifetime values obtained with algal metalloproteins suggest certain differences regarding the relative orientations of the redox partners and/or the distance between them within the electron transfer complex. Actually, the different kinetic behavior observed when comparing spinach Pc and *Monoraphidium* Pc may arise from structural differences both in the hydrophobic and in the hydrophilic patches, in good agreement with the data previously obtained by Bagby *et al.* (1994) with Pc from other algal and higher plants. In addition, the absence of a fast phase when studying the interaction of spinach PSI with cyanobacterial Pc and Cyt suggests that negative charges on the metalloprotein surface are really critical for optimal orientation and electron transfer to take place.

When PSI particles from *Monoraphidium* were used, the fast phase was hardly detected with Pc from either algae or spinach, whereas with *Monoraphidium* Cyt a fast phase with an electron transfer rate constant k_{34} of 8.7×10^4 s^{-1} and 50% in amplitude at high protein concentration was observed (Table 1). This may suggest that the algal PSI has evolved in order to optimize the reaction mechanism of electron transfer from the primitive metalloprotein Cyt, and thus the structural requirements for Pc to yield a similar fast phase with *Monoraphidium* PSI have not yet been settled down in this organism. It is interesting to note that spinach PSI is still able to recognize *Monoraphidium* Cyt as an effective electron donor since the percentage of fast phase is even larger with algal Cyt than with Pc from the same organism (Table 1).

The fast phase in *Anabaena* PSI, which is likewise evident when interacting with Cyt but not with Pc from the cyanobacterium, is significantly faster ($k_{34} = 1.7 \times 10^5$ s^{-1}) than in *Monoraphidium* and spinach PSI particles (Table 1). Such findings suggest not only the existence of evolutionarily divergent pathways, yielding acidic and basic electron donor proteins (Ho & Krogmann, 1984), but also that PSI was able to first optimize its interaction with positively charged metalloproteins by means of long-distance attractive electrostatic interactions between the positive electron donor proteins and negative PSI particles, such interactions being rather repulsive with negatively charged metalloproteins (Tamura *et al.*, 1981; Takabe *et al.*, 1983; Hervás *et al.*, 1992; Medina *et al.*, 1993; Díaz *et al.*, 1994a).

The kinetic analyses reported herein on the slow phase of PSI reduction (either the second phase in kinetics showing fast phase or the only phase in monophasic kinetics) revealed the formation of a stable transient complex between spinach PSI and metalloproteins from spinach, *Monoraphidium*, and *Anabaena*, with the only exception of Pc and Cyt from *Synechocystis* (Table 1). The k_{12} values for complex formation were 2 orders of magnitude higher with the acid donor proteins from spinach and the green algae than with the less acidic or basic proteins from cyanobacteria. Taken together, the above results indicate that charged areas in the surface of the donor proteins are critical for complex formation and electron transfer to PSI. It is also worth noting the interesting compensating effect observed between the equilibrium constants for complex formation (K_A) and complex rearrangement (K_R) in the different PSI/proteins systems exhibiting a fast phase (see Table 1).

Also noteworthy is the role played by magnesium cations in the fast phase of spinach PSI reduction. As previously proposed when analyzing the slow phase (Haehnel *et al.*, 1980b; Hervás *et al.*, 1992, 1994), magnesium cations could act by forming electrostatic bridges between the negative charges in both PSI and donor proteins, thus allowing the PSI/protein complex to reach an optimum orientation for electron transfer to take place. This function appears to be specifically played by divalent cations, as no fast phase could be observed when increasing the ionic strength with sodium chloride up to values comparable to those reached with magnesium chloride (data not shown). For the PSI/Cyt system from *Anabaena*, the effect of $MgCl_2$ on the fast phase suggests that net attractive electrostatic forces between Cyt and PSI make the initially formed complex reach its optimum orientation in such a way that no further rearrangement is required.

Cyt appears to be more efficient than Pc—mainly with regard to the fast kinetic phase—in photosynthetic organisms capable of using both Pc and Cyt as electron donors to PSI. The evolutionary pathway leading to the appearance of the fast phase would involve structural modifications in both PSI and donor proteins, whereas the evolutionary replacement of the ancestral Cyt by Pc as the electron donor to PSI would only require structural changes in PSI; the photosystem would thus have to "learn" how to use Pc with increasing efficiencies as to get into the optimum situation in higher plants. An interesting question still remains to be answered on account of which Pc has been evolutionarily chosen over the ancient Cyt as the electron donor to PSI.

REFERENCES

Arnon, D. I. (1949) *Plant Physiol. 24*, 1−15.

Bagby, S., Driscoll, P. C., Harvey, T. S., & Hill, H. A. O. (1994) *Biochemistry 33*, 6611−6622.

Boardman, N. K. (1971) *Methods Enzymol. 23*, 268−276.

Bottin, H., & Mathis, P. (1985) *Biochemistry 24*, 6453−6460.

Bottin, H., & Mathis, P. (1987) *Biochim. Biophys. Acta 892*, 91−98.

Campos, A. P., Aguiar, A. P., Hervás, M., Regalla, M., Navarro, J. A., Ortega, J. M., Xavier, A. V., De la Rosa, M. A., & Teixeira, M. (1993) *Eur. J. Biochem. 216*, 329−341.

Davis, D. J., Krogmann, D. W., & San Pietro, A. (1980) *Plant Physiol. 65*, 697−702.

Díaz, A., Hervás, M., Navarro, J. A., De la Rosa, M. A., & Tollin, G. (1994a) *Eur. J. Biochem. 222*, 1001−1007.

Díaz, A., Navarro, F., Hervás, M., Navarro, J. A., Chávez, S., Florencio, F. J., & De la Rosa, M. A. (1994b) *FEBS Lett. 347*, 173−177.

Golbeck, J. H. (1992) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 43*, 293−324.
Golbeck, J. H., & Bryant, D. A. (1991) *Curr. Top. Bioenerg. 16*, 83−177.
Gray, H. B., & Malmström, G. (1989) *Biochemistry 28*, 7499−7505.
Haehnel, W., Döring, G., & Witt, H. T. (1971) *Z. Naturforsch. 26B*, 1171−1174.
Haehnel, W., Pröper, A., & Krause, H. (1980a) *Biochim. Biophys. Acta 593*, 384−399.
Haehnel, W., Hesse, V., & Pröper, A. (1980b) *FEBS Lett. 111*, 79−82.
Hatanaka, H., Sonoike, K., Hirano, M., & Katoh, S. (1993) *Biochim. Biophys. Acta 1141*, 45−51.
Hervás, M., De la Rosa, M. A., & Tollin, G. (1992) *Eur. J. Biochem. 203*, 115−120.
Hervás, M., Navarro, F., Navarro, J. A., Chávez, S., Díaz, A., Florencio, F. J., & De la Rosa, M. A. (1993) *FEBS Lett. 319*, 257−260.
Hervás, M., Ortega, J. M., Navarro, J. A., De la Rosa, M. A., & Bottin, H. (1994) *Biochim. Biophys. Acta 1184*, 235−241.
Ho, K. K., & Krogmann, D. W. (1984) *Biochim. Biophys. Acta 766*, 310−316.
Lien, S., & San Pietro, A. (1979) *Arch. Biochem. Biophys. 194*, 128−137.
Mathis, P., & Sétif, P. (1981) *Isr. J. Chem. 21*, 316−320.
Medina, M., Díaz, A., Hervás, M., Navarro, J. A., Gómez-Moreno, C., De la Rosa, M. A., & Tollin, G. (1993) *Eur. J. Biochem. 213*, 1133−1138.
Nanba, M., & Katoh, S. (1983) *Biochim. Biophys. Acta 725*, 272−279.
Quin, L., & Kostic, N. M. (1993) *Biochemistry 32*, 6073−6080.
Rögner, M., Nixon, P. J., & Dinner, B. A. (1990) *J. Biol. Chem. 265*, 6189−6196.
Sandmann, G. (1986) *Arch. Microbiol. 145*, 76−79.
Simondsen, R. P., Weber, P. C., Salemme, F. R., & Tollin, G. (1982) *Biochemistry 21*, 6366−6375.
Sykes, A. G. (1985) *Chem. Soc. Rev. 14*, 283−315.
Takabe, T., Ishikawa, H., Niwa, S., & Itoh, S. (1983) *J. Biochem. (Tokyo) 94*, 1901−1911.
Tamura, N., Itoh, S., Yamamoto, Y., & Nishimura, M. (1981) *Plant Cell Physiol. 22*, 603−612.
Tollin, G., Meyer, T. E., & Cusanovich, M. A. (1986) *Biochim. Biophys. Acta 853*, 29−41.
Van Best, J., & Mathis, P. (1978) *Biochim. Biophys. Acta 503*, 178−188.
Wood, P. M. (1978) *Eur. J. Biochem. 87*, 9−19.
Wood, P. M., & Bendall, D. S. (1975) *Biochim. Biophys. Acta 387*, 115−128.
Yocum, C. F. (1982) in *Methods in Chloroplast Molecular Biology* (Edelman, M., Hallick, R. B., & Chua, N. H., Eds.) pp 973−981, Elsevier Biomedical Press, Amsterdam.
Yoneda, G. S., & Holwerda, R. A. (1978) *Bioinorg. Chem. 8*, 139−159.

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