A Comparative Thermodynamic Analysis by Laser-Flash Absorption Spectroscopy of Photosystem I Reduction by Plastocyanin and Cytochrome c_6 in *Anabaena* PCC 7119, *Synechocystis* PCC 6803, and Spinach†

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Received August 10, 1995; Revised Manuscript Received November 27, 1995⊗

ABSTRACT: A comparative thermodynamic analysis of photosystem I (PSI) reduction by plastocyanin (Pc) and cytochrome c_6 (Cyt) has been carried out by laser-flash absorption spectroscopy in the cyanobacteria *Anabaena* PCC 7119 and *Synechocystis* PCC 6803 as well as in spinach. These three organisms have been reported to exhibit different reaction mechanisms [Hervás, M., Navarro, J. A., Díaz, A., Bottin, H., & De la Rosa, M. A. (1995) *Biochemistry*, *34*, 11321−11326]. Whereas the activation free energy for the overall reaction is mainly enthalpic in nature, long-range electrostatic interactions appear to be attractive in *Anabaena*, but repulsive in *Synechocystis* and spinach. The net interaction between PSI and its two donor proteins in *Anabaena* is similarly affected by ionic strength (the rate constant decreases with increasing salt concentration), but the activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ show different dependencies on ionic strength. A compensation effect between entropy and enthalpy at varying ionic strength is found in all these Pc/PSI and Cyt/PSI systems, except with Cyt and PSI from *Anabaena*. Such a compensation effect is proposed to be mainly due to stabilization of the intermediate electrostatic complex by hydrophobic forces. The electron transfer step seems to be well optimized in the *Anabaena* Cyt/PSI couple, which exhibits a temperature-independent fast kinetic phase and, therefore, a low activation energy barrier. Short-distance forces appear to have gained relevancy in the reaction mechanism of PSI reduction by Cyt and Pc throughout evolution, whereas long-range interactions are prevalent in less evolved organisms.

In higher plants, the type I copper-protein plastocyanin (Pc)[1] acts as a soluble electron carrier between the two membrane-embedded complexes cytochrome b_6-*f* and photosystem I (PSI) (see Chitnis *et al*. (1995), for a review). In some cyanobacteria and green algae, the physiological role of Pc can also be played by the class I *c*-type cytochrome c_6 (Cyt), depending on the availability of copper in the culture medium (Sandmann & Böger, 1980; Wood, 1978). The common physiological role of these two metalloproteins is reflected by their similar sizes and redox potentials (Ho & Krogmann, 1984; Sandmann, 1986). Both Pc and Cyt are acidic in green algae, as is the copper-protein in higher plants, but they can be either neutral, basic, or acidic in cyanobacteria, thereby suggesting that the two evolutionarily unrelated proteins may have co-evolved in response to alterations/mutations in common reaction partners (Ho & Krogmann, 1984).

Whereas the structure and physicochemical properties of Pc are well documented in the literature (see Redinbo *et al*. (1994), for a review), the crystal structure of Cyt from the green algae *Chlamydomonas reinhardtii* and *Monoraphidium braunii* has been solved only recently (Frazão *et al*., 1995a,b; Kerfeld *et al*., 1995). Comparison of the structures of *Monoraphidium* Cyt and *Chlamydomonas* Pc has allowed us to identify regions in the Cyt molecule equivalent to those in Pc, namely, a hydrophobic north pole close to the exposed part of the heme group and an east negative patch (De la Rosa *et al*., 1995; Frazão *et al*., 1995b). Such a structural identity can explain the fact that PSI reduction by either Pc or Cyt isolated from the same organism follows almost identical kinetics. Very recently, the reaction mechanism of electron transfer from these two interchangeable metalloproteins to photooxidized P700 in isolated PSI particles has been studied in a comparative way by laser-flash absorption spectroscopy in a number of different organisms including cyanobacteria, green algae, and higher plants (Hervás *et al*., 1995). As summarized in Scheme 1, 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 transient complex before electron transfer takes place (type III). As a general rule, the type I mechanism should correspond to monophasic reduction of $P700^+$, type II to biphasic reduction, and type III to triphasic reduction. However, with the different protein/PSI systems under study, we have observed monophasic kinetics for the type I and II mechanisms, and biphasic kinetics for the type III mecha-

† This research was supported in part by the Dirección General de Investigación Científica y Técnica (DGICYT, Grant PB93-0922) and Junta de Andalucía (PAI 3182).

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⊗ Abstract published in *Advance ACS Abstracts*, February 1, 1996.

[1] Abbreviations: Cyt, cytochrome c_6; *I*, ionic strength; k_2, bimolecular rate constant; K_A, equilibrium constant for the complex formation reaction; k_{eff}, effective second-order rate constant of the overall reaction; k_{et}, electron transfer first-order rate constant; k_{obs}, observed pseudo-first-order rate constant; K_R, equilibrium constant for rearrangement of redox proteins within the reaction complex; Pc, plastocyanin; p*I*, isoelectric point; PSI, photosystem I; $t_{1/2}$, half-time; $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$, activation free energy, enthalpy, and entropy of the overall reaction, respectively; ΔG_{et}, ΔH_{et}, and ΔS_{et}, activation free energy, enthalpy, and entropy of electron transfer, respectively.

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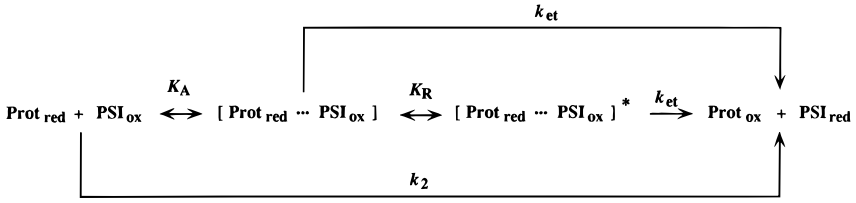
Scheme 1: Proposed Models for the Redox Interaction of Photosystem I (PSI) with either Cytochrome c_6 or Plastocyanin (Prot)[a]



[a] Type I corresponds to an oriented collisional reaction mechanism (k_2); type II requires formation of an intermediate complex (K_A) prior to electron transfer (k_{et}); and type III involves association of the reaction partners (K_A), rearrangement of the resulting complex (K_R), and electron transfer (k_{et}). The asterisk states for the transient complex after rearrangement. See text for explanation.

nism. The proposal has been made that PSI first optimized its interaction with positively charged Cyt and that the evolutionary replacement of the heme-protein by Pc in higher plants involved structural modifications in both the donor protein and PSI (Hervás *et al*., 1995).

The transition state theory has been successfully applied to study the activation parameters for the electron transfer step in redox reactions between inorganic complexes and proteins (Goldberg & Pecht, 1976; Segal & Sykes, 1978), as well as in proteins involved in the photosynthetic electron transfer chain when interacting with their physiological redox partners (Cox, 1975; Takabe *et al.*, 1983; Wood & Bendall, 1975). Bottin and Mathis (1985) have determined the activation parameters for the fast kinetic phase of PSI reduction by Pc in spinach. These studies have shown that changes in entropy usually account for most of the energy barrier of the rate-limiting step.

On the basis of a (minimal) two-step kinetic mechanism involving complex formation followed by intracomplex electron transfer, Díaz *et al.* (1994a) have recently carried out a thermodynamic analysis of Pc and Cyt oxidation by PSI from the green alga *Monoraphidium braunii*. This study allowed us to conclude that the association of the reaction partners to form the transient complex is an exergonic process, and that the following step has to overcome a high energy barrier (*ca.* 70 kJ mol^{-1}) in which the entropic term accounts for most of the energy required (Díaz *et al.*, 1994a). Changes in NaCl concentration and pH induced significant changes in the activation free energy of the overall reaction ($\Delta G^{\ddagger}$) both with Pc and with Cyt, even though the corresponding values for activation enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) underwent changes in opposite directions. A similar compensation effect between enthalpy and entropy has been reported for the reaction of the copper-proteins Pc and azurin with inorganic complexes (Segal & Sykes, 1978).

This paper reports a comparative thermodynamic analysis of Pc and Cyt oxidation by PSI in the cyanobacteria *Anabaena* PCC 7119 and *Synechocystis* PCC 6803 and in spinach, as the three organisms exhibit reaction mechanisms of different types.

MATERIALS AND METHODS

Purification Procedures. Pc and Cyt from *Anabaena* PCC 7119 were purified according to Medina *et al.* (1993). Pc and Cyt from *Synechocystis* PCC 6803 were obtained as described previously (Díaz *et al.*, 1994b; Hervás *et al.*, 1993). Spinach Pc was purified according to Yocum (1982). Protein concentration was determined using absorption coefficients of 4.5 mM^{-1} cm^{-1} at 597 nm for oxidized Pc, and 25 mM^{-1} cm^{-1} at 552 nm for reduced Cyt (Díaz *et al.*, 1994b; Hervás *et al.*, 1993). Spinach PSI particles were obtained upon treatment with digitonin according to Boardman (1971). 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 P700 content in PSI samples was calculated from the photoinduced absorbance increase at 820 nm using an absorption coefficient of 6.5 mM^{-1} cm^{-1} (Mathis & Sétif, 1981). The same batches of PSI and metalloprotein preparations were used throughout these studies.

Laser-Flash Kinetic Spectroscopy. Kinetics of flash-induced absorbance changes in PSI were followed at 820 nm as described previously (Hervás *et al.*, 1995). Unless otherwise stated, the standard reaction mixture contained, in a final volume of 0.2 mL, 20 mM Tricine/KOH (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 Pc or Cyt at the indicated concentrations; at pH 5.5, the buffer used was 20 mM MES/KOH. For the thermodynamic analysis of the so-called fast phase of electron transfer in spinach and *Anabaena* (see the introduction), the reaction mixture was in glycerol/water (1:1). A circulating bath with a water/ethylene glycol (1:1) mixture was used to keep the reaction cell (1 mm path length) thermostated at the desired temperature. To avoid water condensation on the cuvette outer walls at low temperature (<15 °C), the reaction cuvette was placed under a water-free atmosphere by passing a nitrogen gas flow through the sample compartment. Each kinetic trace was the average of 16−20 measurements with 30−45 s spacing between flashes. For most experiments, the estimated error in the observed rate constants (k_{obs}) was ≤10%, based on reproducibility and signal-to-noise ratios. For analyses of the fast kinetic phase with spinach PSI at pH 7.5, errors could be as large as 20%.

Kinetic and Thermodynamic Analyses. Data collection methods were as described previously (Hervás *et al.*, 1995). Oscilloscope traces were treated as sums of several exponential components; exponential analyses were performed using the Marquardt method with the software devised by P. Sétif. Kinetic analyses were carried out according to the reaction mechanisms previously proposed (Hervás *et al.*, 1995). Theoretical thermodynamic parameters were estimated by calculating the electrostatic potential energies of protein−PSI interactions (Watkins *et al.*, 1994) and the rate constants at varying temperature. Entropy−enthalpy compensation was analyzed by comparing the experimental data with those obtained using the Watkins model for electrostatic interactions, in which the rate constants extrapolated at infinite ionic strength were previously fitted to a temperature-

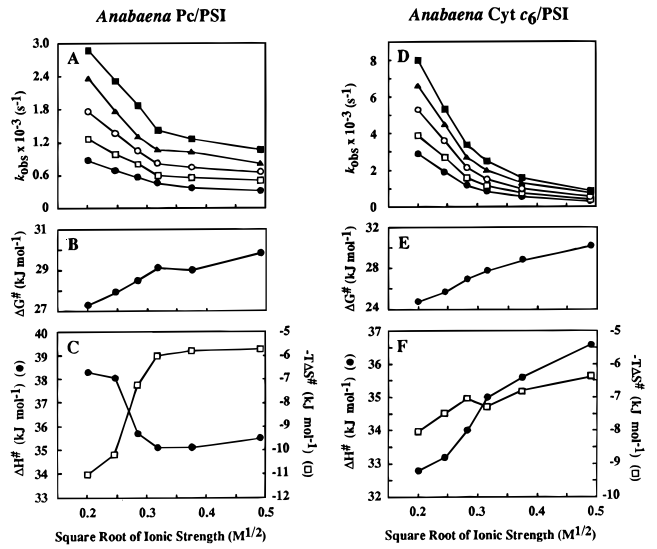


FIGURE 1: Ionic strength dependence of the observed rate constants (k_{obs}) for PSI reduction by plastocyanin and cytochrome c_6 in *Anabaena* at varying temperatures (A, D), as well as effect of the ionic strength on the apparent activation free energy ($\Delta G^{\ddagger}$) (B, E), enthalpy ($\Delta H^{\ddagger}$), and entropy ($\Delta S^{\ddagger}$) (C, F) of the overall reaction. In panels A and D, the temperature values were 278 (●), 283 (□), 288 (○), 293 (▲), and 298 K (■). The thermodynamic parameters in the middle and lower panels were calculated from experimental data in panels A and D at 298 K. Samples were 30 μM in metalloprotein.

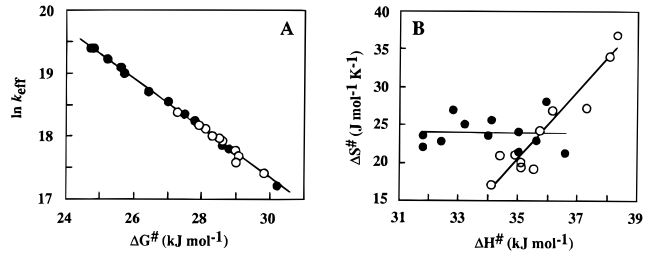


FIGURE 2: Dependence of the effective second-order rate constants (k_{eff}) on the apparent activation free energy ($\Delta G^{\ddagger}$) (A), and correlation between the apparent activation entropy ($\Delta S^{\ddagger}$) and enthalpy ($\Delta H^{\ddagger}$) (B) for PSI reduction by cytochrome c_6 (●) and plastocyanin (○) in *Anabaena*. Experimental data were obtained as in Figure 1 at varying NaCl or $MgCl_2$ concentrations. Units for k_{eff} are M^{-1} s^{-1}.

dependent empirical function (Watkins *et al.*, 1994); the effect of changes in solvent viscosity on the rate constants was thus implicit in the calculations.

RESULTS

Reduction of $P700^+$ in PSI particles from the cyanobacterium *Anabaena* PCC 7119 has been shown to follow different kinetic mechanisms as PSI interacts with either Pc or Cyt. Actually, the laser-flash kinetics of PSI reduction show no fast phase with Pc, but exhibit a sharp fast phase that reaches up to 35% of the total absorbance change with 300 μM Cyt (Hervás *et al.*, 1995). Here we have first studied the effect of ionic strength (I) on the observed pseudo-first-order rate constants (k_{obs}) of both the monophasic kinetics with Pc and the slower phase of the biphasic kinetics with Cyt at varying temperatures (Figure 1A,D). Increasing ionic strength induces a monotonous decrease in k_{obs}, which indicates the existence of attractive electrostatic interactions between the donor proteins and PSI as described previously (Medina *et al.*, 1993). It also becomes clear that the reaction

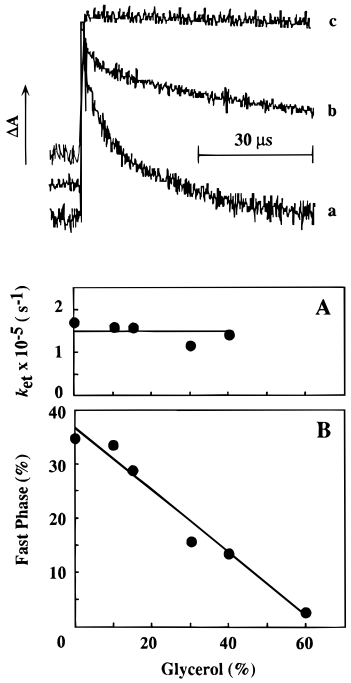


FIGURE 3: Effect of glycerol on PSI reduction by cytochrome c_6 in *Anabaena*. Upper: kinetic traces at 0 (a), 30% (b), and 60% (c) glycerol concentration (v/v). Lower: first-order rate constant (k_{et}) (A) and percentage of fast phase (B) at varying glycerol concentrations. Samples were 150 μM in cytochrome c_6.

rate constant drastically increases with temperature, such an effect being even more evident at low ionic strengths.

The temperature dependence of the effective second-order rate constant (k_{eff}) of *Anabaena* PSI reduction by Pc or Cyt corresponds to linear Eyring plots with no breakpoints (data not shown). From the slope of the lines and the intercept at $1/T = 0$, the apparent enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) values can be determined, which in turn allow calculation of the activation free energy ($\Delta G^{\ddagger}$). The ionic strength dependence of the activation parameters is presented in Figure 1 (middle and lower panels), in which the apparent activation free energy at 298 K increases by 2.5 kJ mol^{-1} for Pc and 5.4 kJ mol^{-1} for Cyt from 0 to 0.2 M NaCl (i.e., from 0.2 to 0.5 $M^{1/2}$ $\sqrt{I}$). It is interesting to note that the change in $\Delta G^{\ddagger}$ is the result of opposite changes in the enthalpic and entropic terms with Pc but not with Cyt. Upon increasing NaCl from 0 to 0.2 M at 298 K with Pc (Figure 1C), the term $-T\Delta S^{\ddagger}$ raises the activation barrier as it increases by 5.3 kJ mol^{-1} (i.e., entropy decreases by 17.8 J mol^{-1} K^{-1}), but this is in part compensated by a parallel decrease of 3.2 kJ mol^{-1} in $\Delta H^{\ddagger}$. Such a compensation effect is not observed with Cyt: the term $-T\Delta S^{\ddagger}$ slightly raises the activation barrier as it increases by 1.6 kJ mol^{-1} (i.e., entropy decreases by 5.5 J mol^{-1} K^{-1}), and $\Delta H^{\ddagger}$ does not decrease but increases by 3.8 kJ mol^{-1} (Figure 1F).

Similar results were obtained with both Pc and Cyt when NaCl was replaced by $MgCl_2$. This finding suggests that Mg^{2+} cations do not play in *Anabaena* the same specific role—formation of magnesium bridges between negatively charged regions—as they play in other organisms (Hervás *et al.*, 1994; Takabe *et al.*, 1983). Figure 2A shows the exponential relation between the effective second-order rate constant (k_{eff}) and the apparent activation free energy ($\Delta G^{\ddagger}$) in *Anabaena*. Most of the changes in activation free energy of PSI reduction by Cyt—but not by Pc—are a consequence of changes in enthalpy rather than in entropy (Figure 2B).

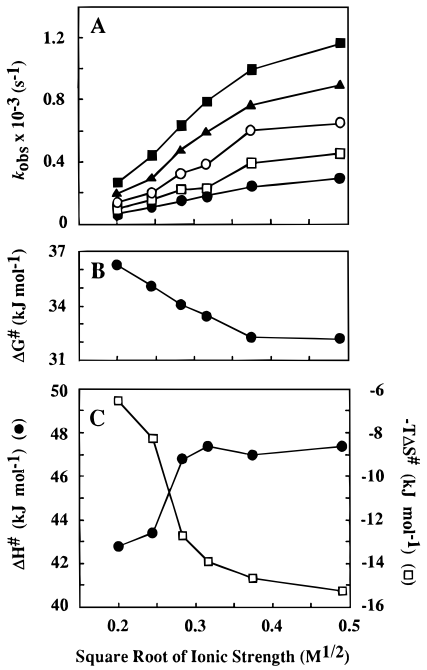


FIGURE 4: Ionic strength dependence of the observed rate constants (k_{obs}) for PSI reduction by cytochrome c_6 in *Synechocystis* at varying temperatures (A), as well as effect of the ionic strength on the apparent activation free energy ($\Delta G^{\ddagger}$) (B), enthalpy ($\Delta H^{\ddagger}$), and entropy ($\Delta S^{\ddagger}$) (C) of the overall reaction. In panel A, the temperature values were 278 (●), 283 (□), 288 (○), 293 (▲), and 298 K (■). The thermodynamic parameters in panels B and C were calculated from experimental data in panel A at 298 K. Samples were 100 μM in cytochrome c_6.

In the *Anabaena* Cyt/PSI system, no temperature effect was found either on the kinetic rate constant of the fast phase or on the contribution of the fast phase to the total absorbance change (data not shown). However, glycerol drastically affects the relative contribution of fast phase, even though the electron transfer rate constant (k_{et}) remains practically unchanged (Figure 3). This effect does not appear to be specific to glycerol as similar results were obtained with dimethyl sulfoxide and ethylene glycol.

A similar thermodynamic analysis has been carried out in the cyanobacterium *Synechocystis* PCC 6803, in which the redox reaction of PSI with the two metalloproteins Pc and Cyt follows a bimolecular mechanism involving formation of an undetected transient complex, i.e., an oriented collisional mechanism in which k_2 is the second-order rate constant of the overall reaction (Hervás *et al*., 1994, 1995) (see also Scheme 1). Figure 4A shows the ionic strength dependence of the observed rate constants for *Synechocystis* Cyt at different temperatures; similar results were obtained with Pc. A significant increase in k_{obs} is observed not only upon increasing the ionic strength—thus indicating the existence of long-distance repulsive electrostatic interactions between the donor proteins and PSI (Hervás *et al.*, 1994)—but also upon increasing the temperature. From the linear Eyring plots of data, the apparent thermodynamic parameters of the overall reaction were determined. As shown in Figure 4B,C, the decrease in $\Delta G^{\ddagger}$ of 4.1 kJ mol^{-1} upon increasing NaCl from 0 to 0.2 M at 298 K is the result of opposite changes in the entropic and enthalpic terms, as $-T\Delta S^{\ddagger}$ decreases by 8.7 kJ mol^{-1} but $\Delta H^{\ddagger}$ increases by 4.6 kJ mol^{-1}. Note that the salt dependences of the terms $\Delta H^{\ddagger}$ and $-T\Delta S^{\ddagger}$ for Cyt and Pc from *Synechocystis* are just reversed as compared to *Anabaena* Pc. Figure 5A shows the exponential relation

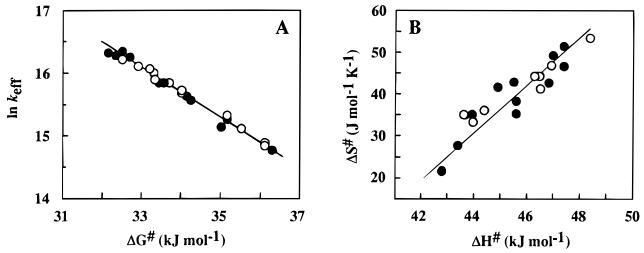


FIGURE 5: Dependence of the effective second-order rate constants (k_{eff}) on the apparent activation free energy ($\Delta G^{\ddagger}$) (A), and correlation between the apparent activation entropy ($\Delta S^{\ddagger}$) and enthalpy ($\Delta H^{\ddagger}$) (B) for PSI reduction by cytochrome c_6 (●) and plastocyanin (○) in *Synechocystis*. Experimental data were obtained as in Figure 4 at varying NaCl or $MgCl_2$ concentrations. Units for k_{eff} are M^{-1} s^{-1}.

Table 1: Apparent Activation Parameters for the Overall Reaction at Varying pH in *Anabaena* and *Synechocystis*[a]

protein	pH	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})	$\Delta G^{\ddagger}$ (kJ mol^{-1})
Anabaena				
plastocyanin	7.5	36.8	29.9	27.9
plastocyanin	5.5	34.9	20.2	28.9
cytochrome c_6	7.5	30.4	11.4	27.0
cytochrome c_6	5.5	29.8	9.5	27.0
Synechocystis				
plastocyanin	7.5	47.4	46.9	33.4
plastocyanin	5.5	47.4	48.5	32.9
cytochrome c_6	7.5	41.8	28.2	33.3
cytochrome c_6	5.5	41.0	29.8	32.1

[a] The buffers used were either 10 mM Tricine/KOH, pH 7.5, or MES/KOH, pH 5.5. Temperature was 298 K.

between the effective second-order rate constant and the apparent activation free energy in *Synechocystis*, according again to the Eyring equation. The apparent activation enthalpy and entropy for the overall reaction are linearly related (Figure 5B).

Table 1 shows the apparent thermodynamic parameters for the overall reaction with Pc and Cyt in *Anabaena* and *Synechocystis* at pH 5.5 and 7.5. The fact that pH has practically no effect on the thermodynamics of PSI reduction makes sense in *Anabaena*, considering that the isoelectric point (p*I*) of the two metalloproteins is quite high (p$I \approx 9$), but it is difficult to explain in *Synechocystis* in which the p*I* of Pc and Cyt is 5.5 (Hervás *et al*., 1995).

A thermodynamic analysis of PSI reduction has also been carried out in spinach, for which Pc is the only electron donor. Previous fast kinetic experiments had revealed the existence of fast and slower phases, with half-times in the range of tens and hundreds of microseconds, respectively. Here we have studied the ionic strength dependence of the second phase at varying temperatures. Plots of the rate constants *versus* ionic strength were bell-shaped curves, with maxima at 50 mM NaCl. The apparent activation free energy of this slower phase decreases by 2.5 kJ mol^{-1} from 0 to 60 mM NaCl, and then increases at higher salt concentrations (data not shown). As is the case in *Monoraphidium* (Díaz *et al*., 1994a) and *Synechocystis* (this work), the change in ΔG is the result of opposite changes in enthalpy and entropy with either NaCl or $MgCl_2$. The thermodynamic analysis of the fast kinetic phase—that is, the electron transfer step itself—at pH 7.5 and 5.5 yields Eyring plots with no breakpoints (Figure 6), from which the values for free energy (ΔG_{et}), enthalpy (ΔH_{et}) and entropy (ΔS_{et}) are calculated

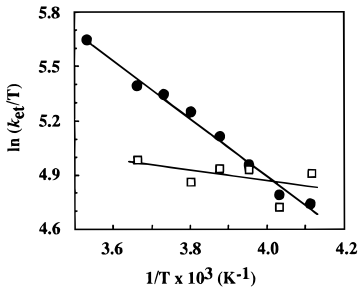


FIGURE 6: Eyring plots for the fast electron transfer from reduced plastocyanin to PSI in spinach at pH 7.5 (□) and 5.5 (●). Samples were 150 μM in plastocyanin. Units for k_{et} are s^{-1}.

Table 2: Effect of pH on the Thermodynamic Parameters for the Fast Phase of Electron Transfer from Plastocyanin to PSI in Spinach[a]

pH	ΔH_{et} (kJ mol^{-1})	ΔS_{et} (J mol^{-1} K^{-1})	ΔG_{et} (kJ mol^{-1})
7.5	4.2	−140	45.9
5.5	13.2	−104	44.2

[a] The buffers used were either 10 mM Tricine/KOH, pH 7.5, or MES/KOH, pH 5.5. Temperature was 298 K.

(Table 2). There is no significant change in ΔG_{et} with pH, but this is again the result of opposite changes in ΔH_{et}, which increases by 9 kJ mol^{-1} when lowering the pH from 7.5 to 5.5, and in the term $-T\Delta S_{et}$, which decreases by 10.7 kJ mol^{-1}.

DISCUSSION

The thermodynamic analyses herein presented on the kinetic mechanism of PSI reduction by Cyt and Pc indicate that the long-range electrostatic interactions between the reaction partners are repulsive in *Synechocystis* and spinach but attractive in *Anabaena*. A compensation effect between entropy and enthalpy at varying ionic strengths has been found in all these protein/PSI systems, with the exception of the *Anabaena* Cyt/PSI couple. This compensation effect has been proposed to be due in other systems to reorganization of water molecules during association of the reaction partners (Edsall, 1935; Tanford, 1973) and/or burying of hydrophobic groups upon complex formation (Rosen & Pecht, 1976; Sturtevant, 1977). In *Anabaena*, the net interaction between PSI and its two donor proteins is affected similarly by ionic strength (k_{obs} decreases with increasing ionic strength), but the activation parameters $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ show different dependences on ionic strength. Actually, the changes in entropy and enthalpy occur in the same direction with Pc (both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ decrease with increasing ionic strength), but occur in opposite directions with Cyt. Even though the ionic strength dependence of $\Delta G^{\ddagger}$ in *Anabaena* is mainly due to the electrostatic nature of the complex between PSI and Pc or Cyt, the factors controlling the electron transfer process differ from each other. With Pc, the electrostatic complex can be stabilized by hydrophobic forces, as proposed by Chothia and Janin (1975). An increase in ionic strength should thus induce a decrease in both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ because of destabilization of the complex and concomitant weakening of hydrophobic forces. With Cyt, however, the absence of any entropy−enthalpy compensation indicates that the electrostatic complex with PSI is stabilized by attractive ionic forces.

The effect of solvent on reaction rates, both for small organic molecules and for protein−protein interactions, involves a certain entropy−enthalpy compensation in such a way that the interaction between the reaction partners shifts the equilibrium between the different structural solvent forms (Jencks, 1987; Parker, 1969). These changes can affect the activation parameters in two ways: first, the structural changes in solvent molecules contribute to changes in the energy required to form the complex; second, changes in the activity of solutes (including the reactants) modify the solvent equilibrium. The latter, i.e., the relaxation term, does not contribute to changes in the total free energy of the reaction but is responsible for parallel changes in enthalpy and entropy. A high compensation effect thus reveals a high participation of the solvent in the reaction. We can then infer from our data that the role played by solvent molecules in the *Anabaena* Cyt/PSI system is rather insignificant and that the changes in activation parameters can only be attributed to attractive electrostatic interactions (see above). Note that the kinetic mechanism of PSI reduction in *Anabaena* seems to be much simpler with Pc than with Cyt, the latter involving rearrangement of the intermediate complex between the donor protein and PSI prior to electron transfer (Hervás *et al.*, 1995).

In the *Anabaena* Cyt/PSI system, neither the reaction rate constant of the fast phase nor the contribution of the fast phase to the total absorbance change was modified in the 259−298 K range, a finding which is in contrast to the results obtained with other redox systems (Mathis *et al.*, 1994; Venturoli *et al.*, 1993). Our data suggest that the electron transfer step is particularly optimized in the *Anabaena* system, which exhibits a low activation energy barrier and a high rate constant ($t_{1/2} = 4$ μs) as compared with the spinach Pc/PSI couple. Actually, the plant system has to overcome a high activation energy barrier and involves long-distance repulsive interactions. The effect of glycerol on the relative contribution of the fast phase in the *Anabaena* Cyt/PSI system suggests that this compound decreases the proportion of effective Cyt/PSI complex but does not alter the electron transfer rate constant within the properly arranged complexes. This would suggest some steric hindrance as glycerol can take the place of water molecules during complex formation; however, the difference in polarity between water and glycerol molecules may also explain the disappearance of the fast kinetic phase at high glycerol concentrations.

The enthalpy−entropy compensation observed in *Synechocystis*, which occurs in the same direction as in *Monoraphidium* (Díaz *et al.*, 1994a), indicates that the intermediate electrostatic complex is stabilized by hydrophobic interactions (see above). As calculated from data in Figure 5B, the slope of the isokinetic line in *Synechocystis* (5.56×10^{-3} K^{-1}) is much higher than that expected from a pure solvent relaxation effect (3.47×10^{-3} k^{-1}) for the temperature range herein considered. Such a difference and the small changes in the activation parameters observed at increasing ionic strength indicate that the hydrophobic forces take part in *Synechocystis* to a lesser extent than in *Monoraphidium*, in which the slope of the isokinetic line (3.42×10^{-3} K^{-1}) is clearly due to the enthalpy−entropy compensation effect (Díaz *et al.*, 1994a). In principle, the slope of the isokinetic lines could be easily explained by assuming contributions of both electrostatic interactions

(estimated to be 14.41×10^{-3} K^{-1} by computer simulation) and solvent effect (for which it should be expected a slope approximately equal to the inverse of the mean temperature value in the range used in the experiments). The slope observed in *Synechocystis* is close to the mean of these two theoretical values. Similar data were obtained with the *Anabaena* Pc/PSI system, in which the experimental value of the slope of the isokinetic line was 4.69×10^{-3} K^{-1} (see Figure 2B), whereas the theoretical slope, assuming a pure electrostatic behavior, was calculated to be 6.86×10^{-3} K^{-1}.

In summary, it can be stated that an enthalpy−entropy compensation effect is clearly observed in most of the Cyt/PSI and Pc/PSI systems analyzed so far. This effect is independent not only of the nature of electrostatic interactions but also of the type of reaction mechanism. The magnitude of the compensation effect differs from one organism to another: It is low in cyanobacteria (in particular with *Anabaena* Cyt and PSI) and comparatively high in green algae and higher plants. This is in good agreement with the role recently proposed for water molecules to play in the control of electron transfer reactions (Berghuis *et al.*, 1994; Casimiro *et al.*, 1993; Meier *et al.*, 1994; Siddarth & Marcus, 1993). However, salts can also modify the reaction rates by controlling the formation of electrostatic complexes rather than the electron transfer step itself. Another alternative would thus be based on the unspecific, stabilizing role played by hydrophobic forces in protein−protein interactions, in which the specificity in molecular recognition is attributed to van der Waals forces and H-bonding (Chothia & Jani, 1975). Unspecific interactions have actually been reported to be critical even in collisional reaction mechanisms (Pontius, 1993). To conclude, short-range forces appear to have gained relevancy in the reaction mechanism of PSI reduction by Cyt and Pc throughout the evolution of oxygenic photosynthetic organisms, whereas long-distance interactions are predominant in less evolved organisms.

ACKNOWLEDGMENT

We thank Prof. Miguel G. Guerrero for critically reading the manuscript, Dr. José M. Ortega for valuable comments, and Mrs. Berta De la Cerda for providing us with *Synechocystis* plastocyanin expressed in *E. coli*.

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BI951876Z