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A comparative study of the thermal stability of plastocyanin, cytochrome c_6 and Photosystem I in thermophilic and mesophilic cyanobacteria

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Abstract

Cytochrome c_6 (Cyt) from the thermophilic cyanobacterium *Phormidium laminosum* has been purified and characterized. It is a mildly acidic protein, with physicochemical properties very similar to those of plastocyanin (Pc). This is in agreement with the functional interchangeability of the two metalloproteins as electron donors to Photosystem I (PS I). The kinetic analyses of the interaction of Pc and Cyt with Photosystem I show that both metalloproteins reduce PS I with similar efficiencies, according to an oriented collisional kinetic model involving repulsive electrostatic interactions. The thermostability study of the *Phormidium* Pc/PS I system compared with those from mesophilic cyanobacteria (*Synechocystis*, *Anabaena* and *Pseudanabaena*) reveals that Pc is the partner limiting the thermostability of the *Phormidium* couple. The cross-reactions between Pc and PS I from different organisms demonstrate not only that *Phormidium* Pc enhances the stability of the Pc/PS I system using PS I from mesophilic cyanobacteria, but also that *Phormidium* PS I possesses a higher thermostability than the other photosystems.

Abbreviations: Cyt – Cytochrome c_6; k_{bim} – bimolecular rate constant for the overall reaction; k_{inf} – bimolecular rate constant extrapolated to infinite ionic strength; Pc – plastocyanin; PS I – Photosystem I; P700 – the photoactive dimeric chlorophyll molecule in Photosystem I; T_i – temperature of inflection of the experimental kinetic data for PS I reduction compared with the Eyring plot; T_m – midpoint temperature of protein thermal transition; T_{max} – temperature at which the maximum rate for PS I reduction is observed; $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ – changes in apparent activation free energy, enthalpy and entropy, respectively, for the overall reaction of PS I reduction

Introduction

In oxygenic photosynthesis, electron transfer between the membrane complexes cytochrome b_6f and Photosystem I (PS I) is accomplished by two diffusable electron carriers, the copper protein plastocyanin (Pc) and the heme protein cytochrome c_6 (Cyt) (see Chitnis 1996; Navarro et al. 1997, for reviews). Higher plants use just Pc, whereas many green algae and cyanobacteria can use either Pc or Cyt depending on the availability of copper in the medium (Merchant and Bogorad 1986; Sandmann 1986; Sandmann et al. 1983). Cyt and Pc are thus functionally interchangeable in species that can express both proteins. Such a convergent evolution of the two electron carriers seems to have occurred in a common ancestor as well as within each organism (De la Cerda et al. 1999; Hervás et al. 1995; Molina-Heredia et al. 2001).

In addition, the structure and function of most of the components of the photosynthetic electron transport chain are similar in cyanobacteria and higher plants (Chitnis 1996; Golbeck 1994), thereby making cyanobacteria an excellent model for the study of oxygenic photosynthesis.

Thermophilic cyanobacteria are the only known organisms having thermostable oxygenic photosynthetic systems, but it is not yet well understood how thermophiles stabilize their photosynthetic apparatus at elevated temperatures (Inoue et al. 2000; Nishiyama et al. 1999). Many investigations in plants have shown that PS I is more stable than Photosystem II at high temperatures (Berry and Björkman 1980; Havaux 1993; Rokka et al. 2000; Sayed et al. 1989; Yordanov et al. 1986). It has been found that moderately high temperatures stimulate PS I activity both *in vivo* and *in vitro* (Armond et al. 1978; Sayed et al. 1994). Studies with spinach Pc have shown that the reduced molecule is more stable (T_m = 71°C) than the oxidized form (T_m = 61 °C) (Gross et al. 1992). Significantly less is known in cyanobacteria on the thermostability of the Pc/PS I and Cyt/PS I systems. The PS I reaction centre has an optimum temperature of ca. 60 °C for Cyt oxidation in *Mastigocladus laminosus*, but this value decreases to 32 °C for the oxidation of the same protein by spinach PS I (Nechushtai et al. 1983). The PS I reaction centre from the thermophilic cyanobacterium *Synechococcus elongatus* is highly stable at elevated temperatures (Sonoike et al. 1990). To the best of our knowledge, there are no reports on the thermostability of Cyt and Pc from cyanobacteria.

In this paper, we present the kinetic and thermodynamic characterization by laser-flash absorption spectroscopy of the interaction of Pc and Cyt with PS I in *Phormidium laminosum*, a filamentous thermophilic cyanobacterium that grows in hot springs at temperatures up to 57–60 °C (Castenholz 1970). The thermal stability of PS I when reacting with the copper protein has been analysed in a comparative way using mesophilic cyanobacteria, namely *Anabaena* sp. PCC 7119, *Synechocystis* sp. PCC 6803 and *Pseudanabaena* sp. PCC 6903.

Materials and methods

Growth conditions

Phormidium laminosum strain OH-1-p clone 1 (courtesy of Dr Juan L. Serra, Bilbao, Spain) was grown in medium D of Castenholz (1970), supplemented with $NaHCO_3$ (0.5 g/l). Cells were grown in 20-l bottles at 50 °C, under continuous illumination by fluorescent tubes, in an atmosphere enriched with 1% CO_2. The production of either Pc or Cyt depended on whether copper was added to or omitted from the culture medium, respectively. *Anabaena*, *Synechocystis* and *Pseudanabaena* were grown autotrophically at 30 °C in a standard BG-11 medium (Rippka et al. 1979).

Purification of plastocyanin and cytochrome c_6

The metalloproteins from *Phormidium* were purified from 150 g of cell paste, as previously described for *Synechocystis* (Hervás et al. 1993) with the following modifications: phosphate was used as buffer instead of Tris, and the proteins were eluted from the DEAE-cellulose column by a 1–100 mM phosphate buffer (pH 7.0) gradient. Protein concentration was determined spectrophotometrically using absorption coefficients of 4.5 mM^{-1} cm^{-1} at 597 nm for oxidized Pc and 25 mM^{-1} cm^{-1} at 553 nm for reduced Cyt. Purity of the resulting protein fractions was determined by using a A_{275}/A_{597} ratio close to 3 for Pc (Schlarb et al. 1999; Varley et al. 1995) and a A_{275}/A_{553} ratio close to 1 for Cyt (Díaz et al. 1994; Molina-Heredia et al. 1998). Pure protein preparations were concentrated and stored at –80 °C. *Pseudanabaena* Pc was purified from the cyanobacterium as described (Hervás et al. 1998). Recombinant Pcs from *Synechocystis* and *Anabaena* were expressed in *Escherichia coli* cells, as previously described (Hervás et al. 1993; Molina-Heredia et al. 1998).

Photosystem I particles

PS I particles were isolated from cells 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 preparations was 130/1, 140/1, 110/1 and 137/1 for *Synechocystis*, *Anabaena, Pseudanabaena* and *Phormidium,* respectively. The P700 content in PS I 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).

Laser-flash absorption spectroscopy

Kinetics of flash-induced absorbance changes in PS I were followed at 820 nm, as described by Hervás et al. (1995). Unless otherwise stated, the standard reaction mixture contained, in a final volume of 0.2 ml, 20 mM buffer (Tricine-KOH, pH 7.5, or MES, pH 5.5), 0.03% β-dodecyl maltoside, an amount of PS I-enriched particles equivalent to 0.36 mg of chlorophyll ml^{-1}, 0.1 mM methyl viologen, 2 mM sodium ascorbate, 10 mM $MgCl_2$, and either Pc or Cyt at the indicated concentration. In the studies of the ionic strength effect, $MgCl_2$ was omitted from the standard reaction mixture, and either NaCl or $MgCl_2$ was added to reach the desired ionic strength. Data collection, as well as kinetic and thermodynamic analyses were carried out as previously reported (Hervás et al. 1995, 1996). Apparent thermodynamic parameters were estimated as in Díaz et al. (1994).

Circular dichroism spectroscopy

CD analyses were performed in a thermostated Jasco spectropolarimeter, model J-710. A cell with 1 cm path-length was used for all measurements.

Fluorescence spectroscopy

Fluorescence spectra were recorded using a Perkin-Elmer fluorescence spectrophotometer, model LS-5. The excitation wavelength was 275 nm, and light emission was recorded between 280 and 500 nm. The temperature was monitored by a Digitron 2008 thermocouple, fitted to a flexible probe inserted directly into the 1-cm path-length cell. The average emission of fluorescence from 289 to 350 nm was considered in order to reduce the background noise. Data were corrected for the sloping of the baselines for the folded and unfolded proteins, normalized to describe the fraction of folded protein, and fitted to a two-state equilibrium mechanism for protein unfolding to estimate the values for T_m (Privalov 1979).

Gel electrophoresis

Isoelectric points were determined by electrofocusing gel electrophoresis in 5% acrylamide–0.2% bisacrylamide gels, with an ampholyte mixture from pH 2.5 to 8 and IEF markers ranging from 3.6 to 9.3 (Robertson et al. 1987).

Molecular mass was determined by SDS-PAGE using a 16% acrylamide–0.5% bisacrylamide running gel (Schägger and von Jagow 1987). The molecular mass marker MW17SDS from Sigma was used as a standard kit.

Redox titrations

Redox titrations were performed in a dual-wavelength spectrophotometer setup as described by Ortega et al. (1988). The differential absorbance changes at 597 nm *minus* 500 nm for Pc, and at 553 nm *minus* 570 nm for Cyt, were monitored in the presence of the following redox mediators: menadione, diaminodurol and *p*-benzoquinone, at 20 μM final concentration.

Results

Characterisation of Phormidium *cytochrome* c_6

Cyt from *Phormidium* has been purified and characterised. The resulting physicochemical data are summarised in Table 1, along with those previously reported for *Phormidium* Pc (Schlarb et al. 1999). The molecular mass of Cyt determined by SDS-PAGE is similar to that inferred from the gene sequence (Table 1), a value that is comparable to that of Pc. Cyt is a slightly acidic protein, with an isoelectric point (pI) of 5.10 similar to that of *Phormidium* Pc (Schlarb et al. 1999; Stewart and Kaethener 1983), as well as to those of Pc and Cyt from *Synechocystis* (Hervás et al. 1994). *Phormidium* Cyt has a redox potential value of +331 mV (Table 1), equivalent to that determined for Pc by Schlarb et al. (1999). Such close analogies between Cyt and Pc from *Phormidium* have previously been observed in many other organisms (Díaz et al. 1994; Hervás et al. 1993, 1998; Molina-Heredia et al. 1998), a fact that is consistent with the same physiological role played by both metalloproteins and their parallel evolution (Navarro et al. 1997).

Kinetic analysis of Photosystem I reduction

The observed pseudo first-order rate constant (k_{obs}) for the reduction of photooxidized P700 in *Phormidium* PS I particles at different temperatures depends linearly on the concentration of added metalloprotein – either Pc (Figure 1) or Cyt (not shown). In all cases, the kinetics can be well fitted to single exponential curves with no fast phase. The interaction of both Pc and Cyt with PS I does thus follow an oriented collisional kinetic model involving no formation of any

Table 1. Physicochemical properties of *Phormidium* cytochrome c_6 and plastocyanin

Protein	Molecular mass (kDa)		pI	$E_{m,pH\,7.0}$ (mV)
	SDS-PAGE	Gene sequence		
Plastocyanin	11.3 (± 0.5)	11.42[a]	4.96 (± 0.09)	+336 (± 5)
Cytochrome c_6	8.9 (± 0.5)	9.04[b]	5.10 (± 0.08)	+331 (± 5)

[a]Schlarb et al. (1999).
[b]Personal communication from J. Wastl (Cambridge, UK).

Table 2. Bimolecular rate constant and apparent activation parameters for the overall-reaction of PS I reduction by plastocyanin and cytochrome c_6 from *Phormidium*, *Synechocystis* and *Anabaena*

	pH	$k_{bin}\times10^{-7}$ ($M^{-1}\ s^{-1}$)	$k_{inf}\times10^{-7}$ ($M^{-1}\ s^{-1}$)		$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J $mol^{-1}\ K^{-1}$)	$\Delta G^{\ddagger}$ (kJ mol^{-1})
			$MgCl_2$	NaCl			
Phormidium							
Plastocyanin	7.5	1.17	1.73	1.46	48.1	52.8	32.3
	5.5	1.35			48.1	53.9	32.0
Cytochrome c_6	7.5	1.57	1.29	1.37	40.8	30.3	31.8
	5.5	1.90			40.1	29.0	31.4
Synechocystis							
Plastocyanin	7.5	0.86[a]	1.09[c]	1.06[a]	47.4[f]	46.9[f]	33.4[f]
	5.5	0.99[a]			47.4[f]	48.5[f]	32.9[f]
Cytochrome c_6	7.5	0.89[b]	1.10[c]	1.30[b]	41.8[f]	28.2[f]	33.3[f]
	5.5	1.40[b]			41.0[f]	29.8[f]	32.1[f]
Anabaena							
Plastocyanin	7.5	7.6[d]	0.59[d]	0.59[d]	36.8[f]	29.9[f]	27.9[f]
	5.5	5.3			34.9[f]	20.2[f]	28.9[f]
Cytochrome c_6	7.5	11.3[e]	1.14[e]	1.14[e]	30.4[f]	11.4[f]	27.0[f]
	5.5	11.5			29.8[f]	9.5[f]	27.0[f]

All the parameters were determined at 25 °C. Values obtained from: [a]De la Cerda et al. (1997); [b]De la Cerda et al. (1999); [c]De la Cerda (1998); [d]Molina-Heredia et al. (2001); [e]Molina-Heredia et al. (1999); [f]Hervás et al. (1996).

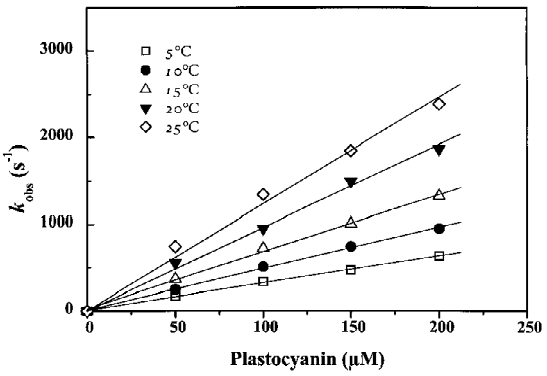

Figure 1. Dependence upon plastocyanin concentration of the observed rate constant (k_{obs}) for PS I reduction in *Phormidium* at varying temperature and pH 7.5. Other experimental conditions were as described under 'Materials and methods'.

kinetically detectable transient complex, as is the case in other organisms (Hervás et al. 1995).

The bimolecular rate constants (k_{bim}) for the overall reaction of PS I reduction by Cyt or Pc can be calculated from linear plots as those in Figure 1. As can be seen in Table 2, which summarizes the k_{bim} values in *Phormidium* along with those previously reported in *Anabaena* and *Synechocystis*, both at pH 5.5 and 7.5, Pc and Cyt are equally efficient in reducing PS I within each cyanobacterium and at any pH.

Thermodynamic parameters

From the Eyring equation it is possible to calculate values for the changes in apparent activation enthalpy ($\Delta H^{\ddagger}$), entropy ($\Delta S^{\ddagger}$) and free energy ($\Delta G^{\ddagger}$) for the overall reaction of PS I reduction. Table 2 summarizes the estimated values for these three thermodynamic

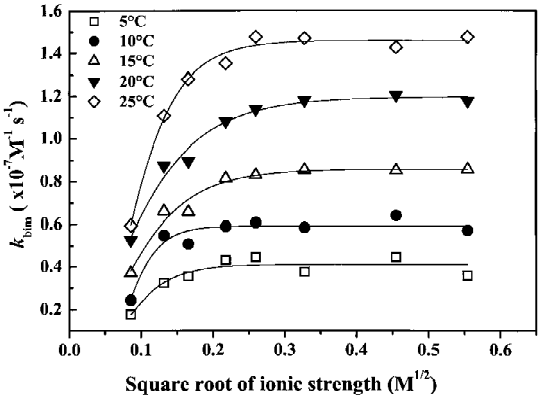

Figure 2. Ionic strength dependence of the bimolecular rate constant (k_{bim}) for PS I reduction by plastocyanin in *Phormidium* at different temperatures and pH 7.5. Pc concentration was 100 μM. The ionic strength was increased by adding small amounts of a concentrated solution of NaCl. Solid lines correspond to theoretical fits according to the Watkins equation (Watkins et al. 1994).

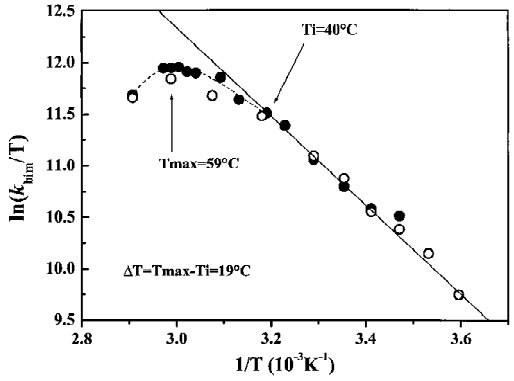

Figure 3. Eyring plots for PS I reduction by either plastocyanin (closed circles) or cytochrome c_6 (open circles) in *Phormidium* at pH 7.5. T_{max} denotes the temperature at which the maximum rate is reached, and T_i is the temperature at which the inflection between the Eyring prediction and experimental data takes place.

parameters in *Phormidium*, *Synechocystis* and *Anabaena*. The $\Delta G^{\ddagger}$ values are not significantly different from one another organism, either with Pc or Cyt at pH 7.5 and 5.5. Within the same, however, the differences in $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ with Pc and Cyt (Cyt always shows lower values than Pc for both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$) reflect their structural dissimilarity, which are compensated as to make the changes in apparent free energy nearly identical.

Ionic strength dependence

Taking into account the electrostatic nature of the interaction between PS I and its electron donor proteins, the bimolecular rate constants for PS I reduction by both Pc (Figure 2) and Cyt (not shown) were determined at varying ionic strength and temperature. By increasing the ionic strength, the k_{bim} values with Pc increase and reach a nearly limiting value at different temperatures. This can be explained by assuming that the repulsive electrostatic interactions between Pc and PS I are weakened at high ionic strength, a fact that is observed with Cyt as well. A similar ionic strength dependence is observed when $MgCl_2$ was used instead of NaCl, thereby suggesting that the *Phormidium* Pc/PS I system, unlike that from other organisms (Hervás et al. 1995), is not specifically affected by magnesium cations.

Using the formalism developed by Watkins et al. (1994), it is possible to extrapolate the bimolecular rate constant to infinite ionic strength (k_{inf}), which provides a better understanding of the intrinsic reactivity of the redox partners in the absence of electrostatic interactions. As shown in Table 2, the estimated values for k_{inf} with Pc and Cyt are similar in the three cyanobacteria herein considered, namely *Phormidium*, *Synechocystis* and *Anabaena*.

Effect of temperature on PS I and plastocyanin

The Eyring plots in Figure 3 show the kinetic constants of *Phormidium* PS I reduction by Pc and Cyt within a wide temperature range. The close superposition of experimental data with both metalloproteins makes evident their equivalent physiological function and biophysical features. The experimental points for Pc and Cyt are linear in the Eyring plots at temperatures below 40 °C, but they are nonlinear at higher temperatures. The temperature at which the experimental data separate from the Eyring line is here called inflection temperature (T_i). The data can be fitted to a polynomial curve that bends at temperature values higher than T_i, reaches a maximum at 59 °C (T_{max}) and decreases thereafter.

The two parameters, T_i and T_{max}, were also determined for the Pc/PS I system from *Synechocystis*, *Anabaena* and *Pseudanabaena*. As can be seen in Table 3, the value for T_{max} is higher in the thermophile *Phormidium*, as would be expected, than in the mesophilic cyanobacteria. Among the mesophiles, the *Anabaena* system has the highest T_{max} value, whereas that of *Pseudanabaena* is more sensitive to temperature than the others. It is interesting to note that the value for T_i in *Phormidium* is lower than expected from the thermophilic nature of the organism.

Table 3. Activation energy (E_a), maximum temperature (T_{max}) and inflection temperature (T_i) for the reduction of PS I by plastocyanin from different sources at pH 7.5

	Photosystem I											
	Phormidium			*Synechocystis*			*Anabaena*			*Pseudanabaena*		
Plastocyanin	E_a (kJ mol^{-1})	T_{max} (°C)	T_i (°C)	E_a (kJ mol^{-1})	T_{max} (°C)	T_i (°C)	E_a (kJ mol^{-1})	T_{max} (°C)	T_i (°C)	E_a (kJ mol^{-1})	T_{max} (°C)	T_i (°C)
Phormidium	38.3	59	40	–	–	–	39.0	59	41	51.6	61	41
Synechocystis	–	–	–	42.3	51	40	–	–	–	–	–	–
Anabaena	28.9	61	40	39.3	54	46	33.6	54	46	–	–	–
Pseudanabaena	33.3	46	30	–	–	–	–	–	–	31.2	49	30

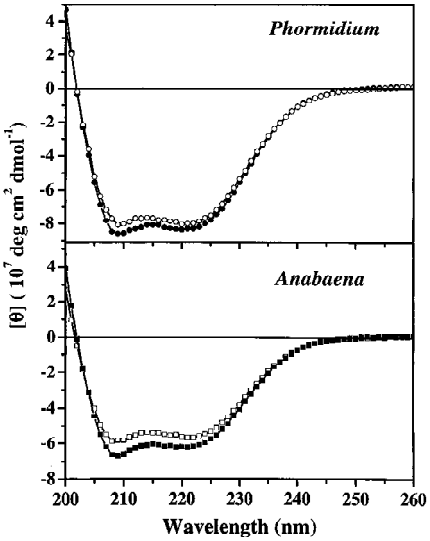

Figure 4. Effect of temperature on the far-UV circular dichroism spectra of PS I. Spectra were recorded at 25 °C, either before (closed symbols) or after (open symbols) heating the sample for 5 min at 70 °C. Experiments were carried out in 10 mM phosphate buffer, pH 7, supplemented with 0.03% β-dodecyl maltoside. PS I concentration was 42 nM (*Phormidium*) or 51 nM (*Anabaena*).

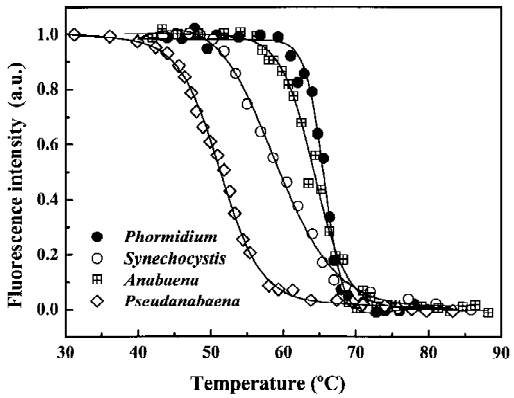

Figure 5. Thermal denaturation of cyanobacterial plastocyanins as monitored by following their fluorescence changes. Excitation was carried out at 275 nm, and the fluorescence intensity was measured as described under 'Materials and methods'. Experiments were run in 50 mM phosphate buffer, pH 7.5. Solid lines correspond to theoretical fits to a simple two-state unfolding model (Privalov 1979). Other experimental conditions were as described under 'Materials and methods'.

The cross-reactions between Pc and PS I from different organisms, which were likewise investigated (Table 3), suggest that the thermal properties of the copper protein, rather than PS I, are those that determine the heat resistance of the different Pc/PS I couples. The activation energy (E_a) for every reaction was indeed estimated from the Arrhenius plots (Table 3), with the cross-reaction between *Phormidium* Pc and *Pseudanabaena* PS I yielding the highest values for E_a and T_{max}. In addition, high T_{max} values (ca. 60 °C) are obtained for Pc and PS I cross-reactions between *Phormidium* and *Anabaena* (Table 3).

The thermostability of PS I from *Phormidium* and *Anabaena* was probed by circular dichroism (CD). The CD spectra of native PS Is (Figure 4) was typical of α-helical proteins, as expected from the X-ray structure of a homologous PS I (Krauss et al. 1996). After 5 min at 70 °C, the CD spectra recorded at 25 °C reveal an irreversible decrease in helical content that is significantly less pronounced in *Phormidium* than in *Anabaena* because of the higher thermostability of the former.

The thermal unfolding of Pc from *Phormidium*, *Anabaena*, *Synechocystis* and *Pseudanabaena* was monitored by fluorescence spectroscopy. Figure 5 shows the normalised data fitted to a two-state model in order to calculate T_m values. Pc exhibits the lowest midpoint value for the thermal transition in

Pseudanabaena (T_m = 51.2 °C), followed by *Synechocystis* (T_m = 57.7 °C), whereas *Anabaena* exhibits a T_m value of 64.1 °C, which is close to the highest value of 65.6 °C that was found in *Phormidium*.

Discussion

The data presented in this work indicate that the interaction of both Pc and Cyt with PS I from *Phormidium* follows a simple oriented collisional reaction mechanism, with no formation of any kinetically detectable transient complex, as previously observed in other cyanobacteria (Hervás et al. 1994). Such a kinetic model is based on the repulsive electrostatic interactions between the two redox partners, as experimentally inferred from the ionic strength dependence of the rate constant. Actually, the kinetic behaviour of the Pc/PS I and Cyt/PS I systems is identical in *Phormidium* (this work) and *Synechocystis* (Hervás et al. 1996), whose metalloproteins display the same pI value. Indeed, the slight increase in the bimolecular rate constant at acid pH for *Phormidium* (Table 2), which was also observed in *Synechocystis*, is in good agreement with the slightly acidic pI of the metalloproteins, as well as with the pH value of 5.8–6.5 for the thylakoid lumen under illumination (Kramer et al. 1999).

The apparent thermodynamic parameters for PS I reduction with Pc and Cyt in *Phormidium*, *Synechocystis* and *Anabaena* are independent of pH. The heme protein shows lower values than Pc for both $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$, but these values compensate each other to give similar values for $\Delta G^{\ddagger}$. These differences between Pc and Cyt can be ascribed to their structural dissimilarities. Actually, the lower value of $\Delta H^{\ddagger}$ with Cyt suggests a higher number of favourable interactions in the transition state, whereas the lower value of $\Delta S^{\ddagger}$ with Cyt indicates the release of less solvent molecules during the eventual formation of the transient complex.

The electron transport machinery of the photosynthetic membranes is very sensitive to elevated temperatures. Stewart and Bendall (1980) have reported that the thermal stability of O_2 evolution in growing cultures of *Phormidium* is apparently not retained when the cells are broken to yield membrane fragments. The results reported herein show that the Pc/PS I system from *Phormidium* is more thermostable than those from the mesophiles *Anabaena*, *Synechocystis* and *Pseudanabaena*. The T_{max} value for the redox activity of the Pc/PS I and Cyt/PS I systems from *Phormidium* (59 °C) is close to that estimated for the Cyt/PS I couple from the thermophilic cyanobacterium *Mastigocladus laminosus* (Nechushtai et al. 1983).

Few data are available on the thermostability of PS I, in which P700 is the most stable redox centre with a temperature for 50% inactivation of ca. 70 °C in spinach (Shuvalov 1976; Takamiya and Nishimura 1972) and 68 °C in pea (Hoshina et al. 1989). Even less is known in cyanobacteria. The P700 molecule is destroyed by treatment at temperatures above 80 °C for 5 min, with a half time inactivation temperature of 93 °C in *Synechococcus elongatus* (Sonoike et al. 1990). The Fa/Fb, Fx centres of PS I exhibit temperatures for 50% inactivation of 53 and 65 °C, respectively, in spinach, and 70 °C each in *Synechococcus elongatus*. Our comparative study by CD spectroscopy of PS I from *Phormidium* and *Anabaena* shows a higher thermostability of *Phormidium* PS I, a fact that is also made evident in the cross-reaction between *Phormidium* PS I and *Anabaena* Pc.

The only available data on thermal denaturation of Pc come from spinach, with a T_m value of 71 and 61 °C for the reduced and oxidized state, respectively (Gross et al. 1992). Not only do the redox state and pH affect the thermal stability, but the electrostatic charge at the lipid bilayer does as well (Taneva et al. 2000). In cyanobacteria, there are no data other than those presented herein for the oxidized form of the copper protein from *Anabaena*, *Synechocystis*, *Pseudanabaena* and *Phormidium*. The estimated T_m values indicate that Pc is more stable in *Phormidium* than in the other cyanobacteria, but is similar in *Phormidium* and in spinach. This finding makes it difficult to explain how Pc itself can confer thermostability to the *Phormidium* Pc/PS I system. It is interesting to note that the T_m values of several Pcs are lower than those of P700 (see above), a fact suggesting that the limiting factor in the thermostability of the Pc/PS I system is the copper protein. The cross-reactions herein presented reveal that *Phormidium* Pc enhances the heat resistance of the Pc/PS I system when using PS I from several different mesophilic cyanobacteria. On the other hand, the observed thermolability of *Pseudanabaena* Pc agrees with the lower T_{max} and T_i values observed both with its own PS I and with that from *Phormidium*.

To conclude, we can say that *Phormidium* Pc and PS I are more thermostable than their homologues in other cyanobacteria, but these differences are not enough to confer the required thermostability to the

Pc/PS I system of a thermophilic cyanobacterium. The basis for the thermostability of the *Phormidium* Pc/PS I system might thus involve additional features in its surroundings, including lipids and other proteins on the lumenal side.

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