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Proline 14 is impairing the electron transfer from plastocyanin to photosystem I in the prochlorophyte *Prochlorothrix hollandica*

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Abstract. Plastocyanin (Pc) from the prochlorophyte *Prochlorothrix hollandica* exhibits some peculiar differences at its north hydrophobic patch as compared with Pc from other organisms. Actually, residues at positions 12 and 14 are tyrosine and proline, respectively, in *Prochlorothrix*, but they are glycine and leucine in all others. Photosystem I (PSI) reduction by single (Y12G, Y12F, Y12W and P14L) and double (Y12G/P14L) mutants of *Prochlorothrix* Pc has been investigated by laser flash absorption spectroscopy. With all the mutants, the observed pseudo first-order rate constant reaches a saturation plateau at high Pc concentration, as is the case with wild-type (WT) Pc, suggesting the formation of a transient Pc-PSI complex. The equilibrium constant for complex formation is not significantly altered by mutations, but the electron transfer rate constant is drastically changed, in particular by mutations at position 14. Pc mutants have also been used as donor proteins in experiments with PSI isolated from different organisms (cyanobacteria and higher plants). The experimental data reveal that reversion of the “exclusive” Pro-14 of *Prochlorothrix* Pc to the "standard" leucine enhances the reactivity of Pc towards PSI.

Introduction

Plastocyanin (Pc) is a small redox protein (molecular mass, ca. 10.5 kDa) that functions in photosynthesis as a mobile electron carrier between cytochrome b_6f and Photosystem I (PSI) complexes (see Navarro *et al.*, 1997 for a review). Whereas higher plants produce just Pc, there is a number of cyanobacteria and eukaryotic algae that are able to produce cytochrome c_6 as an alternative carrier under copper defficiency (Navarro *et al*., 1997). The interaction between these two metalloproteins and PSI has been studied by laser-flash absorption spectroscopy in a wide variety of evolutionarily differentiated organisms, allowing us to propose different reaction mechanisms for PSI reduction (Hervás *et al*., 1995).

A recent study has shown that *Prochlorothrix* Pc reacts with PSI according to a two-step reaction mechanism involving complex formation and electron transfer, the complex being mainly hydrophobic in nature. Cyt, in its turn, follows a three-step reaction mechanism with rearrangement of redox partners within an intermediate electrostatic complex prior to electron transfer (Navarro *et al*., 1999). So much different kinetic mechanisms reflect interesting differences not only in electrostatic surface charge distribution but also in dynamic properties.

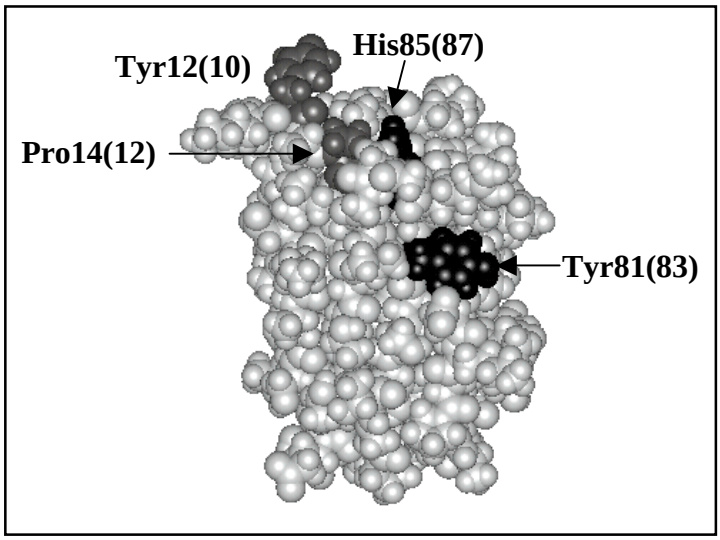

Fig. 1. Space-filling model of *Prochlorothrix* plastocyanin showing the residues modified by mutagenesis. The molecule is oriented with its typical east face (electrostatic area around Tyr81; site 2) to the right, whereas the north hydrophobic patch (or site 1) is at the top. The mutant residues are depicted in gray, and His85 and Tyr81 in black. The numbering corresponding to spinach Pc is shown between brackets.

Despite the relatively low number of conserved residues shared with other Pcs, the *Prochlorothrix* molecule has a similar overall folding pattern (Babu *et al*., 1999). Interestingly, *Prochlorothrix* Pc has an altered hydrophobic patch, a region that is thought to be crucial in Pc interaction with its redox counterparts. The presence of two unique residues (Tyr12 and Pro14 in *Prochlorothrix versus* Gly10 and Leu12 in all other Pcs) yields a structurally different hydrophobic surface, with Tyr12 extending outwards from this patch (Babu *et al*., 1999) (Figure 1).

In this paper, we extend our previous studies of *Prochlorothrix* PSI reduction by WT Pc to analyze the reactivity of Pc mutants at Tyr12 and Pro14. The kinetic data herein reported indicate that the replacement of Pro14 by leucine — which is the "standard" residue in all other Pcs — makes the copper protein react much more efficiently with PSI.

Materials and Methods

The construction of mutant genes and purification of mutant proteins were as previously described (Babu *et al*. 1997). PSI particles from the different organisms were obtained by β-dodecyl maltoside solubilization as described in Hervás *et al*. (1995).

Kinetics of flash-induced absorbance changes in PSI were followed at 820 nm as previously described (Hervás *et al*., 1995). Unless stated otherwise, the experimental setup and programmes used in the analyses were as in Hervás *et al*. (1995).

Results and Discussion

The kinetic analysis herein reported with a number of Pc mutants from the prochlorophyte *Prochlorothrix hollandica*, which exhibits a peculiar hydrophobic patch, has allowed us to infer interesting conclusions on the role of some specific residues at the hydrophobic patch of Pc in its interaction with PSI.

The laser-flash induced kinetic traces of PSI reduction by WT and mutated Pcs are monoexponential, even at high donor protein concentration (not shown). The dependence of the observed pseudo first-order rate constant (k_{obs}) upon donor protein concentration shows a saturation profile (Fig. 2, left). This finding suggests the formation of a bimolecular transient [Pc-PSI] complex prior to electron transfer, as previously described for the WT system (Navarro *et al.*, 1999). As can be seen in

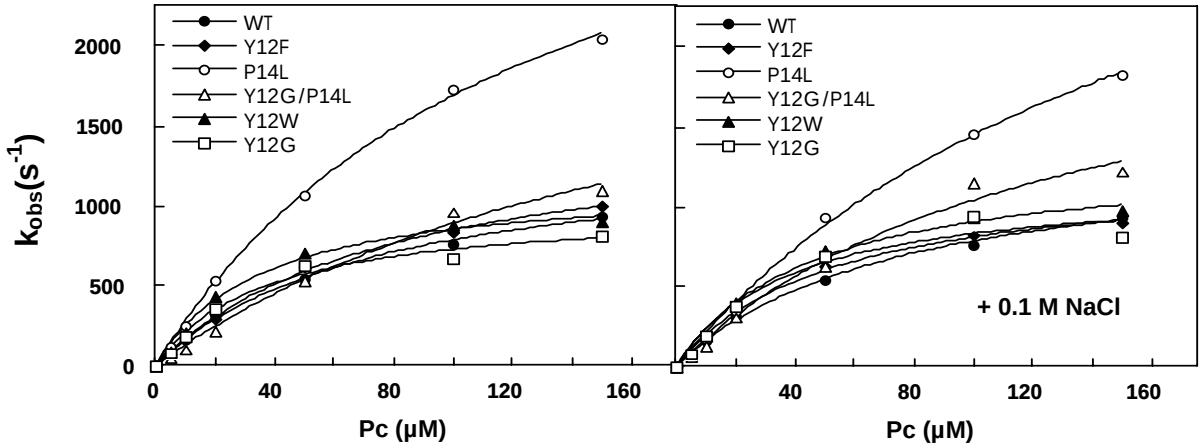

Fig. 2. Dependence upon Pc concentration of the observed rate constant (k_{obs}) for PSI reduction by WT and mutant Pc in the absence (left) and in the presence (right) of 0.1 M NaCl. The reaction cell contained: 20 mM Tricine-KOH pH 7.5, 10 mM $MgCl_2$, 100 μM methyl viologen, 2 mM sodium ascorbate, 0.03% ß-dodecyl maltoside and 1.7 μM P700. Temperature was 25 ^{o}C.

Table 1, there are no significant changes in K_A with all mutants. This is in contrast with the results obtained from mutations at the north pole of other Pcs, from which it has been inferred that both the hydrophobic and charged patches are involved in the interaction of Pc with PSI (Sigfridsson, 1998). To check if the hydrophobic nature of the interaction between Pc and PSI in *Prochlorothrix* (Navarro *et al*., 1999) is altered by mutations, the kinetics of PSI reduction were followed at varying ionic strength (Figure 2, right). As can be seen in Table 1, none of the mutants shows changes in their reactivity upon increasing salt concentration, as previously described for the WT Pc (Navarro *et al*., 1999).

Concerning the electron transfer step, Table 1 shows that none of the mutations at position 12 alters significantly the efficiency of Pc in donating electrons to PSI. The only exception is the replacement of tyrosine with glycine, which induces a decrease of about 30% in k_{et}. These results suggest that in *Prochlorothrix* Pc, unlike in other Pcs, the residue at position 12 (corresponding to Gly10 in poplar Pc) plays only a minor role in hydrophobic interactions, if any. Much more drastic is the effect obtained by replacing proline at position 14 with leucine, as the k_{et} value increases up to three times with respect to the WT species (Table 1). This can be explained by assuming that Pro14 is in some way distorting the interaction site, its replacement thus making the copper site be ~1 Å closer to the acceptor.

Table 1. Midpoint redox potential (E_m) of wild-type and mutant Pcs, as well as association rate constant (K_A) and electron transfer rate constant (k_{et}) for PSI reduction by the different copper proteins.

Protein	E_m (mV)	$K_A x10^{-4}$ (M^{-1})		k_{et} (s^{-1})	
		– NaCl	+ 0.1 M NaCl	– NaCl	+ 0.1 M NaCl
WT	370	1.4	1.6	1,390	1,450
Y12G	367	2.7	2.5	1,000	1,150
Y12F	368	1.5	2.0	1,500	1,200
Y12W	n.d.	2.8	2.0	1,160	1,300
P14L	355	0.9	0.6	3,900	4,050
Y12G/P14L	356	0.6	0.8	2,600	2,400

In previous work, we have described that *Prochlorothrix* WT Pc does not form any electron transfer complex with PSI from either spinach or the cyanobacteria *Anabaena* and *Synechocystis*, the copper protein showing a very low reactivity in all these cross reactions (Navarro *et al*., 1999). As the mutants constructed in this study are aimed to revert the "exclusive" hydrophobic patch of *Prochlorothrix* to the "standard"

configuration, we have also checked the reactivity of mutants towards heterospecific PSI. In all cases, linear dependencies were observed when plotting the observed rate constants *versus* protein concentration (not shown). Replacement of Pro14 with leucine makes the bimolecular rate constant of PSI reduction — both with eukaryotic and prokaryotic photosystems — increase by one order of magnitude. Again, the mutants at Tyr12 did not change significantly their reactivity. These results are in contrast with the previously proposed requirement for a flat surface in the area of Gly12 at the hydrophobic patch of Pc to ensure efficient electron transfer to PSI (Hippler *et al*., 1996), thus indicating that *Prochlorothrix* Pc presents an altered interaction area with PSI as compared with other Pcs.

The fact that WT Pc from *Prochlorothrix* possesses a residue that is impairing its redox interaction with its physiological electron acceptor confirms that this organism is using a divergent protein, which appeared before evolution "discovered" that a leucine at position 14 enhances the reactivity of Pc. To conclude, we can say that reversion of Pro14 to the "standard" leucine makes *Prochlorothrix* Pc much more reactive towards PSI from any organism, including *Prochlorothrix*.

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