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Conformational changes of plastocyanin induced by redox state or metal substitution: Effect on electron transfer to photosystem 1

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Introduction

In our attempts to understand the mechanism of electron transfer (ET) between plastocyanin (Pc) and photosystem 1 (PS1) we are using flash-photolysis kinetics to monitor the oxidation state of P700. The decay of the absorption at 830 nm (which reflects the ET from Pc to PS1) is at least biphasic. Two different models can account for this behavior: the conformational change model (*CC-model*) [Bottin & Mathis, 1985, Sigfridsson et al., 1996] or the reversible ET model (*RE-model*) [Drepper et al., 1996]. Our studies of the ionic-strength dependence of the kinetics favor the *CC-model* [Olesen et al., 1999].

Earlier PAC studies [Danielsen et al., 1999] have shown that Ag⁺-Pc binds much stronger to PS1 than Cd²⁺-Pc and since these derivatives resemble the reduced and the oxidized Pc, respectively, this suggests that there might be a structural difference between the two states. If Cu⁺-Pc binds stronger to PS1 than Cu²⁺-Pc, then the reduction potential of bound Pc will be increased, which will favor the *RE-model* [Drepper et al., 1996].

We have used Ag⁺-Pc, Cd²⁺-Pc and Zn²⁺-Pc as competitive inhibitors to Cu⁺-Pc in flash-photolysis studies to determine the dissociation constant for the binding of these metal-substituted forms to PS1. In addition, we will report on preliminary X-ray crystallographic studies aimed at finding out if there are any structural differences between reduced and oxidized Pc that could lead to different dissociation constants.

Materials and methods

Wild-type (recombinant) spinach Pc was expressed and purified from *Escherichia coli* as described in [Ejdeback et al., 1997]. To get Zn²⁺-Pc, the CuSO₄ in the growth culture and in all buffers was substituted with ZnSO₄. Substitution for Ag and Cd was carried out anaerobically using potassium cyanide as chelator for the copper-ion. The apo-Pc was then subjected to a ten-fold excess of AgNO₃ or Cd(NO₃)₂ for approximately one hour to get Ag⁺-Pc or Cd²⁺-Pc, respectively. Excess of metal was removed by desalting on a gel-filtration column. PS1 with Chl a / Chl b = 6.8 was prepared from spinach as previously described [Boardman et al. 1971].

Crystallization of the Glu8Asp mutant of spinach Pc was carried out at room temperature in 100 mM Na-acetate (pH 4.5), 31 - 34 % PEG 3350 and 0.10 - 0.25 M MgCl₂ using the hanging drop technique [Xue et al. 1998]. The facilities at the MAX II synchrotron was used for high-resolution data collection at low temperature (100 K) of both the oxidized and reduced states of the protein.

Results and discussion

Flash illumination of a mixture of Cu^+ -Pc and PS1 results in a rapid absorption increase at 830 nm, due to photooxidation of P700^{red} , which is followed by a decay that reflects the ET from Cu^+ -Pc (Fig.1, blue trace). When all P700^{ox} has been reduced, there remains an absorption from Cu^{2+} -Pc (18%) which decays on a longer time scale.

Addition of Ag^+ -Pc results in a much slower reduction of P700^{ox} (Fig.1, red and black) while a further addition of Cu^+ -Pc accelerates the decay kinetics (not shown). This indicates that Ag^+ -Pc is able to compete with Cu^+ -Pc in the binding to PS1 and that the inhibition is reversible. From the effect on the kinetics we estimate that the redox-inert Ag^+ -Pc binds equally good to PS1 as Cu^+ -Pc.

The effect of Zn^{2+} -Pc and Cd^{2+} -Pc (not shown) is much weaker. Even a large excess of these derivatives over Cu^+ -Pc results only in slightly slower decay kinetics (Fig.2, red and blue traces). We find that the dissociation constants for Cd^{2+} -Pc and Zn^{2+} -Pc are $310 \mu\text{M}$ and $440 \mu\text{M}$ respectively. Comparing these results with the dissociation constant of Cu^+ -Pc, $K_{\text{red}}^{\text{diss}} = 28 \mu\text{M}$ [Olesen et al., 1999, using the *RE-model*] shows that Ag^+ -Pc binds equally well as Cu^+ -Pc while Cd^{2+} -Pc and Zn^{2+} -Pc binds 10 and 15 times weaker, respectively. If these derivatives can be considered to be good structural models for Cu^+ -Pc and Cu^{2+} -Pc, respectively, then these results favor the *RE-model*.

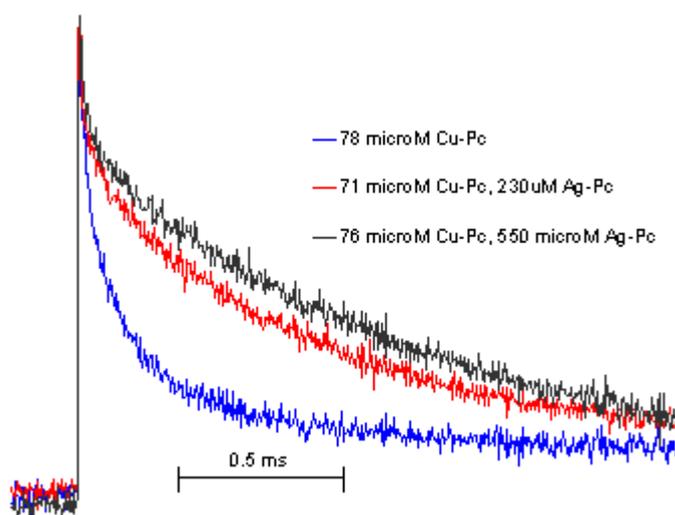


Fig. 1 Absorption transients at 830 nm of photooxidized P700 in the presence of Cu^+ -Pc and variable amounts of Ag^+ -Pc. Each sample contained $10 \mu\text{M}$ PS1 particles, 20 mM Tris (pH 7.5), 0.1 mM methyl viologen, 2 mM Na-ascorbate and 7 mM MgCl_2 in a cuvette of 1 mm thickness. Each trace is the average of 4 flash-induced transients recorded with a spacing of 20 s .

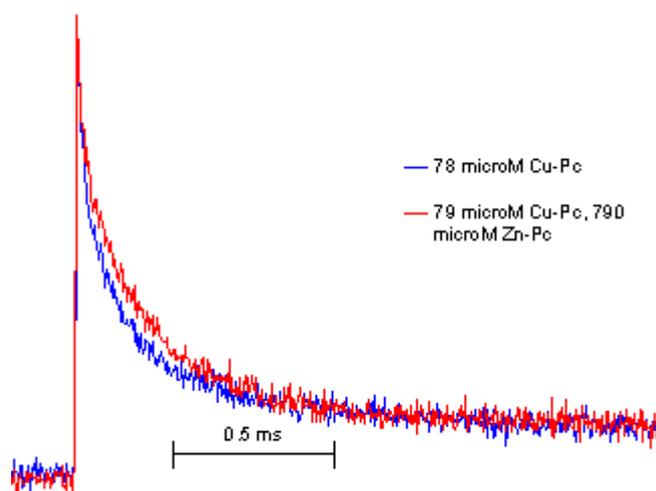


Fig. 2 Absorption transients at 830 nm of photooxidized P700 in the presence of Cu^+ -Pc and variable amounts of Zn^{2+} -Pc. See Fig. 1 for experimental conditions.

Figure 3 shows details of the electron density around the Cu-site for Cu^+ -Pc at a resolution of 1.15 Å. Some residual electron density can be seen in the vicinity of the Cu site, which probably can be attributed to a minor conformation. If one compares this structure with that of Cu^{2+} -Pc at a similar high resolution and the same pH 4.5, there are only significant differences around the Cu-site (Fig. 4). In particular the distance between the Cu ion to His-87 decreases. Similar changes were previously observed for reduced poplar Pc when increasing the pH from 3.8 to 7.8 [Guss et al., 1986]. From our data it is clear that His-87 remains coordinated to the oxidized Cu ion at low pH. For the rest of the protein there does not seem to be any significant differences between the two redox states of spinach Pc.

The redox changes that we observe in the Cu-site extend to the hydrophobic surface at the top of the molecule which is known to make contact with PS1 [Olesen et al., 1999 and references therein]. Therefore, these structural differences between oxidized and reduced Pc could be related to their different affinities to PS1.

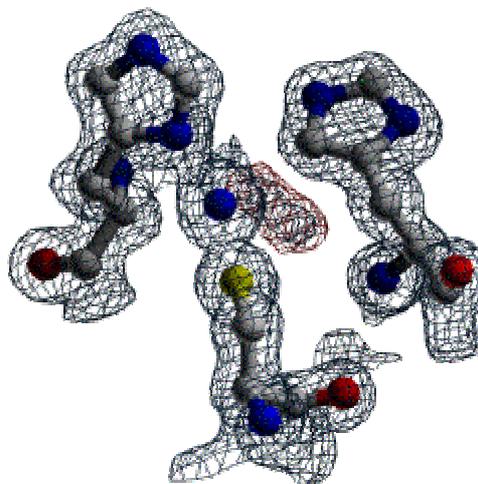


Fig. 3 Cu site of the preliminary structure of reduced spinach Pc. A 2Fo-Fc electron density map contoured at 1.0 σ is shown in grey.

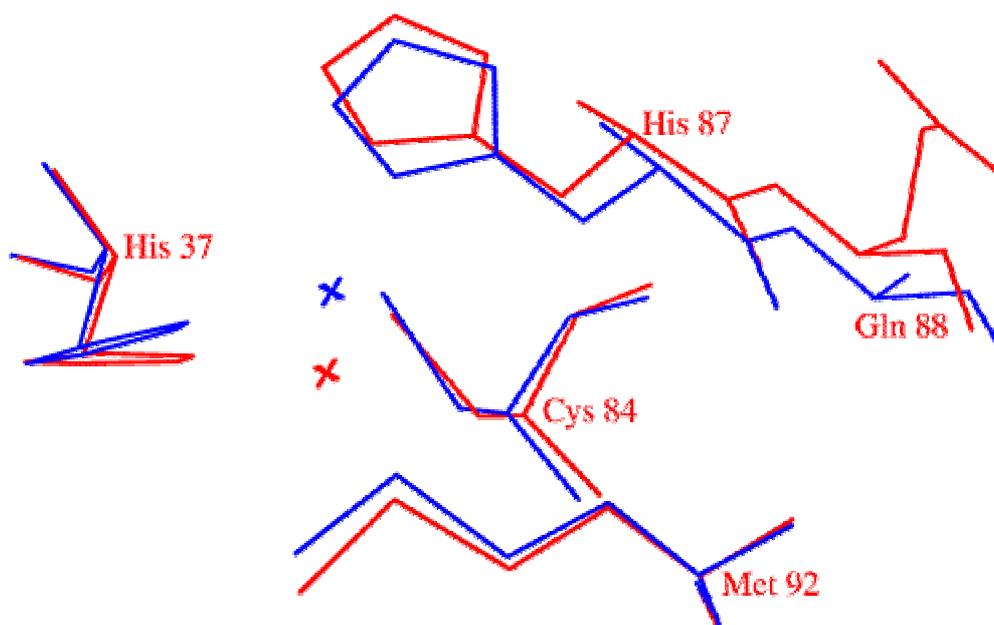


Fig. 4 Cu-sites of high resolution oxidized (blue) and reduced (red) Pc (obtained by soaking the crystal in ascorbate). RMSD for all C α atoms 0.19 Å. Significant shifts are only seen in the Cu site and neighbouring residues (0.3 - 0.6 Å).

Acknowledgements

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