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Competitive inhibition of electron donation to photosystem 1 by metal-substituted plastocyanin

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ABSTRACT

The electron transfer from wild-type spinach plastocyanin (Pc) to photosystem 1 has been studied by flash-induced absorption changes at 830 nm. The decay kinetics of photo-oxidized P700 are drastically slower in the presence of Ag(I)-substituted Pc, while addition of Zn(II)-substituted Pc has a weaker effect. The metal-substituted forms of Pc act as competitive inhibitors of the reaction between normal, Cu-containing, Pc and P700. The inhibition constants obtained from an analysis of the kinetic data were 30 and 410 μM for Ag(I)- and Zn(II)-substituted Pc, respectively. When the Gly8Asp mutant form of Pc was used instead of the wild-type form, the corresponding values were found to be 77 and 442 μM. If the Ag- and Zn-derivatives can be considered as structural mimics of reduced and oxidized CuPc, respectively, our results imply that there is a redox-induced decrease in the affinity between Pc and photosystem 1 that follows the electron donation to P700. Our data also imply that the Gly8Asp mutation can diminish the magnitude of this change. The findings reported here are consistent with a reaction mechanism where the electron transfer in the complex between Pc and photosystem 1 is assumed to be reversible.

1. Introduction

Plastocyanin (Pc) is a small (10.4 kDa), soluble blue copper protein that transfers electrons from cytochrome b_6/f to photosystem 1 (PS1) in the photosynthetic electron-transfer (ET) chain. Pc contains a single type 1 copper site, where the Cu ion is liganded by two histidines, one methionine and one cysteine, see Fig. 1. This type of coordination results in a characteristic blue colour for the oxidized protein. See [1] for a recent review of Pc and other type-1 Cu proteins. There are three areas on the protein that are important for the interaction with the redox partners: the hydrophobic, northern end, where the copper is situated, and two acidic patches surrounding Tyr83 on the eastern side. The ET from cytochrome b_6/f to Pc [2–6] and from Pc to PS1 [7–14] are both thought to be mediated by the hydrophobic patch via the Cu ligand His87 and the acidic patches probably serve to guide the protein into a proper position for ET. Earlier work in our group has dealt with mutational studies of these patches in order to establish their role in the reaction mechanism [10–14]. In the present publication we address the question of whether the state of the metal ion affects the binding of Pc to PS1. This is done with a novel approach where redox-inert metal-substituted forms of Pc are used as competitive inhibitors of the flash-induced ET from normal Pc to PS1.

The interaction between Pc and PS1 is commonly studied by flash-induced absorption spectroscopy. The ET reaction is initiated with a flash to photooxidize P700 (the reaction-centre chlorophyll of PS1) and the ET is monitored through absorption changes of the latter. Stopped flow is another common technique in studies of biological redox reactions. But, in contrast to stopped flow, the flash-induced technique can provide information about the ET in the reactive complex since this is allowed to form before the start of the measurement.

The reduction of photo-oxidized P700 is at least biphasic for Pc and PS1 from plants and green algae. There is a fast phase with a concentration-independent rate constant (k_f) of approximately $8 \cdot 10^4\ s^{-1}$ and a slower phase with an observed first-order rate constant (k_s) that depends on the Pc concentration. In a simple interpretation, k_f reflects the ET in the reactive complex while k_s corresponds to the rate of complex formation. However, details in the kinetics indicate a more intricate reaction mechanism [12–17]. In particular, the simple mechanism cannot explain why the relative amplitude of the fast phase saturates at approximately 75% at high Pc concentrations and why k_s saturates at a value approximately 25% of that of k_f.

Bottin and Mathis suggested originally that the observed kinetics can be explained by a rate-limiting conformational change that occurs in the Pc–PS1 complex before the ET takes places [15]. In this model the ET is assumed to be irreversible since the reduction potentials of free Pc and P700 are quite different, 384 and 490 mV [10], respectively. However, Drepper et al. [16] found that the reduction potential of Pc shifts to 420 mV upon binding to PS1 and suggested an alternative model where the ET is reversible and no conformational change takes

Abbreviations: ET, electron transfer; Pc, plastocyanin; PS1, photosystem 1

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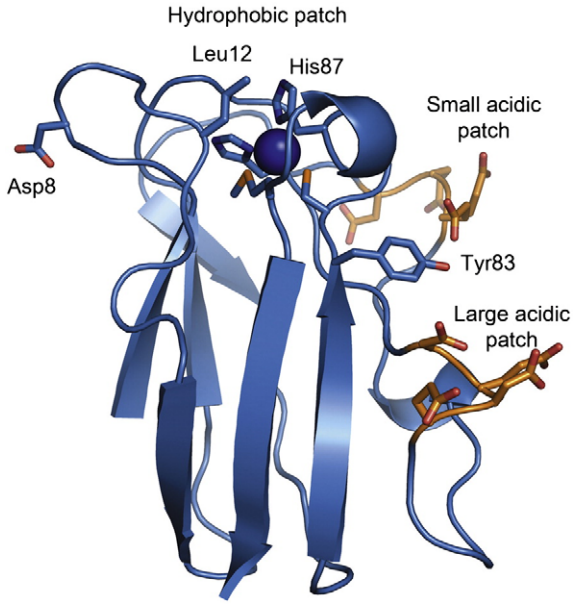

Fig. 1. Structure of the oxidized spinach plastocyanin mutant G8D at pH 7.5 [21]. The Cu site is located below the hydrophobic patch at the "northern" end, while the acidic patches are situated on the "eastern" side of the protein. The Cu ligands (only His87 is labelled), Asp8, Leu12 and Tyr83 as well as the acidic-patch residues are represented with sticks. The figure was drawn with PyMol.

place in the complex. As shown by Olesen et al. [17], both models account equally well for the observed kinetics, but the interpretation of the observed rate constants in terms of mechanistic rate constants is different in the two models.

If a reversible ET is assumed, then a driving force for ET in the Pc–PS1 complex (ΔE^0_c) of 30–40 mV can be deduced from the kinetic data [17]. This driving force is connected to the difference in reduction potential between free P700 and Pc (ΔE^0_f) through the following thermodynamic relation:

$$\frac{RT}{F}\ln\frac{K'_{diss}}{K_{diss}} = \Delta E^0_f - \Delta E^0_c \quad (1)$$

Here, K_{diss} and K'_{diss} are the dissociation constants for the Pc–Ps1 complex before and after the ET, respectively. Thus, with the reduction potentials and driving force given above, one expects that ET from Pc to P700 weakens the binding to PS1 with a factor of 15–20. This is of course attractive from a physiological perspective, since this would increase the turnover of PS1.

That oxidized Pc (Pc^{ox}) may bind weaker to PS1 than reduced Pc (Pc^{red}) has been supported by kinetic studies [16] and perturbed angular correlation (PAC) spectroscopy [18]. In the PAC studies, Ag(I)-substituted Pc (AgPc) was used to mimic Pc^{red} and Cd(II)-substituted Pc (CdPc) was used as a mimic of Pc^{ox}. The dissociation constant for CdPc from PS1 (with reduced P700) was found to be 24 times larger than for AgPc based on the effect on the rotational-correlation time of Pc upon adding PS1 [18].

The dissociation constants K_{diss} and, in particular, K'_{diss} are difficult to obtain from kinetic data. In the present work we show in a novel way how they can be estimated from the influence of metal-substituted Pc on the flash-induced kinetics of the reaction between normal Pc (CuPc) and PS1. We have used AgPc as a mimic of Cu(I)Pc and Zn(II)-substituted Pc (ZnPc) to mimic Cu(II)Pc. We have found that these redox-inert forms of Pc act as competitive inhibitors of the reaction between CuPc and PS1 but with widely different inhibition constants. Similar studies, using metal-substituted Cu proteins as competitive inhibitors of normal ET reactions, have been made in the past: ET from cytochrome *c*-550 to amicyanin [19] and from cytochrome b_6/f complex to Pc [20]. However, these studies employed steady-state and stopped-flow kinetics, respectively, rather than the flash-photolysis technique used here and, in both previous cases, the Cu protein (amicyanin and Pc, respectively) acts as an electron acceptor.

The present studies were made both with wild-type spinach Pc, Pc(WT), and with the G8D mutant form of the protein, Pc(G8D) (Fig. 1). The reason for using the latter is that there are small but significant differences in its reaction with PS1 [13] that need to be further clarified, in particular since crystallographic studies of spinach Pc are made with this mutant and not with Pc(WT), which so far has not been possible to crystallize [21,22].

2. Materials and methods

2.1. Protein purification and metal substitution

Wild-type spinach Pc and the G8D mutant protein were expressed and purified as described by Jansson et al. [23]. PS1 was prepared as described by Sigfridsson et al. [12].

Ag-substituted Pc was obtained in the following way: Highly purified Pc with an absorption ratio A_{278}/A_{597} of less than 1.2 and at a concentration of approximately 1.7 mM in 10 mM Tris (pH 8.0) was reduced with 5 mM Na-dithionite and desalted on a prepacked PD-10 column. The column contained Sephadex® G-25 (Amersham Pharmacia) pre-equilibrated with 20 mM Hepes (pH 7.5), 0.5 M Na_2SO_4, 0.1 mM EDTA and 2 mM β-mercaptoethanol. The Pc-containing fractions, as determined by absorbance measurements, were pooled and incubated on ice for 30 min with 50 mM KCN (from a 2.5 M stock solution) to remove the Cu ion. The high ionic strength provided by the Na_2SO_4 avoids the unfolding of apoPc [24]. The absorption at 597 nm was recorded under oxidizing conditions during the KCN treatment to validate that no Cu was left in the Cu site at the end of the treatment. Cu ions and KCN were removed by a desalting step on another PD-10 column pre-equilibrated with 20 mM Hepes (pH 7.5) and 0.5 M Na_2SO_4. $AgNO_3$ was added at a tenfold excess over apoPc and then the sample was incubated on ice for 1 h followed by concentration. In order to remove unspecifically bound Ag ions, 7 mM $MgSO_4$ was added and then the sample was desalted again. The whole procedure was performed under reducing and anaerobic conditions with N_2-saturated buffers in a glove box to avoid oxidation of the sulphur-containing amino acids in the Cu-site. Chloride-free buffers were used throughout the procedure to avoid precipitates of AgCl (s).

For production of ZnPc, the protein was expressed and purified as for normal recombinant CuPc [23] except that $ZnSO_4$ was added in the growth media and purification buffers instead of $CuSO_4$.

The success of the metal substitution was verified by nano-spray quadropole-time of flight (Q-TOF) mass spectrometry performed at the Institute of Biomedicine at University of Gothenburg.

2.2. Flash-photolysis kinetics

The ET from Pc to PS1 was studied by monitoring the absorbance changes due to flash oxidation of P700 in PS1 and subsequent reduction of P700 by Pc. The oxidation of P700 was obtained by a flash from a Nd:YAG laser (Spectra Physics GCR 190-10, wavelength of 532 nm, pulse duration of 8 ns, pulse energy of 5 mJ/cm^2). The photooxidation and subsequent reduction of P700 was monitored at 829 nm by a continuous-wave diode laser (Melles-Griot 06DLD203) and a home-built detector (Si photodiode and preamplifier) connected to a digital oscilloscope (Tektronix TDS 3032). PS1 at a concentration of 10 or 6 μM (for Pc(WT) and Pc(G8D), respectively) was mixed with buffer, CuPc and Ag or ZnPc in a cuvette with a 1.4 mm optical path length. The buffer consisted of 20 mM Tris (pH 7.5), 7 mM $MgCl_2$, 2 mM sodium ascorbate and 0.1 mM methyl viologen. Sodium ascorbate keeps CuPc reduced between the flashes and methyl viologen serves as an electron acceptor to PS1. 10–20 different concentrations of CuPc were used, ranging from 20 to 800 μM. Metal-substituted Pc was used at the following concentrations: AgPc at 0, 0.2 and 0.5 mM and ZnPc at 0, 0.75 and 1.1 mM. For each combination of Pc concentrations, four flash-induced absorption transients were averaged with a spacing of 20 s between the flashes. In addition, the transients were recorded on two different time scales, 40 and 200 ms, with 500 data points for each scale, in order to monitor both fast and slow components in the kinetics.

2.3. Theory

As noted in the Introduction, two different reaction mechanisms have been proposed to account for the multiphasic behaviour of the reduction of P700 by Pc in higher plants. In the following we will use the model of Drepper et al. [16], which can be written:

$$Pc^{red} + PS1^{ox} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} Pc^{red}\cdot PS1^{ox} \underset{k_{-et}}{\overset{k_{et}}{\rightleftharpoons}} Pc^{ox}\cdot PS1^{red} \xrightarrow{k'_{off}} Pc^{ox} + PS1^{red}$$

Here, k_{off}, k_{et}, k_{-et} and k'_{off} are first-order rate constants (unit: s^{-1}) while k_{on} is a second-order rate constant (unit: $M^{-1}\,s^{-1}$) and *red* and *ox* denote reduced and oxidized species, respectively. The analysis by Olesen et al. [17] shows that this reaction model leads to three decaying exponentials in the reduction kinetics of $P700^{ox}$. However, in the limits of